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SOURCE AND SIGNIFICANCE OF STREPTOCOCCI IN MARKET MILK.

By F. S. JONES, V.M.D.

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(Received for publication, January 28, 1920.)

Considerable discussion has arisen as to the source and significance of streptococci in milk. Most of the observations were made before the use of the blood agar plate method or before the use of more accurate determinations of acid production became a common practice. New interest in the problem has been aroused since the outbreaks of septic sore throat attributed to contamination of the milk supply with streptococci pathogenic for man. A more thorough knowledge of milk streptococci, particularly those producing hemolysis in blood agar plates, is necessary. The source of such organisms is of particular interest.

Rogers and Dahlberg[1] undertook the study of milk streptococci in detail. They examined mixed milk, udder milk, feces, and saliva of cows. Blood agar plates were not used. The bouillon employed was prepared from beef extract to which dextrose, lactose, saccharose, mannite, raffinose, starch, and glycerol were added to make 1 per cent solutions. In all a large number of cultures were studied. The fecal streptococci were characterized by their ability to attack the sugars, including raffinose, and their inability to utilize alcohols. The salivary strains fermented dextrose, lactose, saccharose, mannite, and frequently raffinose. The amount of acid produced was lower than that obtained from the fecal streptococci. They divided the udder streptococci into two groups on the basis of the liquefaction of gelatin. One group, which they considered indistinguishable from *Streptococcus pyogenes*, fermented dextrose, lactose, and saccharose with an occasional culture breaking down mannite, starch, or inulin. In addition to liquefying gelatin, the other group attacked dextrose, lactose, saccharose, mannite, and generally fermented glycerol. From these studies the conclusion was reached that the streptococci found in market milk were of udder origin.

[1] Rogers, L. A., and Dahlberg, A. O., *J. Agric. Research*, 1913–14, i, 491.

Davis[2] in examining the market milk from nine dairies found hemolytic streptococci in eight samples; eight of the samples had been pasteurized. One of the pasteurized samples did not contain hemolytic streptococci. In the other seven they made up from 5 to 45 per cent of the flora. In one sample of certified (raw) milk 40 per cent of the bacteria were hemolytic streptococci. The data concerning fermentation are given for ten strains. They are compared with three strains of human origin and one culture isolated from a case of bovine mastitis. All the milk streptococci fermented dextrose, lactose, maltose, and salicin. Seven acidulated saccharose and one mannite. The titratable acidity in dextrose bouillon varied from 5.05 to 6.55 per cent. Two of the human cultures produced somewhat less acid than the milk strains (4.55 and 4.70 per cent), but the other produced more acid (6.25 per cent). The mastitis strain could not be differentiated by its cultural characters from the milk streptococci. However, it possessed considerable pathogenicity for rabbits. Davis points out that of the 85 strains studied none had properties which would justify his considering them of human origin. He concludes by stating that the milk strains are different from certain strains of hemolytic streptococci found at times in the diseased udders of cows, since the latter are virulent for rabbits and are from human sources.

The writer[3,4] has shown that apparently normal cows may harbor in the udder, streptococci which are identical in all cultural characters and agglutination affinities with those causing mastitis. It was pointed out that in a herd in which a large number of cows suffered from mastitis many cows carried streptococci in the udder. From this observation it was assumed that many such streptococci would gain access to the milk supply.

To establish the possible types of streptococci which may appear in market milk, examinations of the vaginal discharges, saliva, feces, and skin of cows in a large herd were undertaken. The milk from this herd is of high quality and is sold in one large city as certified milk. In another city it is marketed as Grade A raw milk which is certified by a board of physicians. The usual precautions taken in the production of certified milk are maintained.

The Possible Sources of Streptococci.

In a previous paper[4] the vaginal streptococci were considered in detail. At that time it was noted that hemolytic streptococci were not found on the normal vaginal mucosa. A considerable number

[2] Davis, D. J., *J. Infect. Dis.*, 1916, xix, 236.
[3] Jones, F. S., *J. Exp. Med.*, 1918, xxviii, 253.
[4] Jones, F. S., *J. Exp. Med.*, 1918, xxviii, 735.

of the non-hemolytic variety were isolated and studied. They fell into two principal groups, those attacking dextrose, lactose, saccharose, maltose, and mannite, and those fermenting the first four sugars but not fermenting mannite. Salicin fermentation predominated in each group.

Swabs were employed in isolating streptococci from the mouth and skin. The swabs were introduced into the mouth and rubbed over the tongue and mucous membranes. Within an hour they were agitated in 10 cc. of sterile 0.9 per cent sodium chloride solution. From this suspension three platinum loopfuls were inoculated into 12 cc. of melted 2 per cent agar prepared from veal infusion which had been cooled to 45°C. The mixture was plated with 1 cc. of defibrinated horse blood. The plate cultures were examined after incubation for 24 hours at 38°C.

For the skin examinations the lumbar region was chosen. Since the cows were confined in stanchions the possibility of contaminating this area with either feces or saliva was not considered great.

Feces were obtained as early after defecation as possible and suspended in the usual amount of salt solution and plated at once.

The cultures were inoculated into tubes containing 13 cc. of fermented veal infusion broth (+ 0.6 to +0.8 phenolphthalein) to which the test substances were added to make a 1 per cent solution. Titrations were made after 5 days incubation at 38°C.

In all, the saliva, skin, and feces of 45 cows were examined. Hemolytic streptococci have not been isolated from these regions. The saliva contains many streptococci. In all, thirty-seven strains have been isolated and studied. They comprise, however, an exceedingly heterogeneous group. Mannite fermentation is frequent and the proportion of raffinose and inulin fermenters is high. As a rule, low acid production in dextrose is characteristic. A tabulation of their characters has been omitted as it will be shown later that such streptococci have not been found in the milk.

From the skin thirteen cultures of streptococci have been isolated. Their cultural characters, given in Table I, are more uniform than those from the mouth. From Table I it will be noted that the characteristic streptococcus from the skin is a type which produces short chains in bouillon, acidulates milk, and ferments dextrose, lactose,

saccharose, maltose, raffinose, mannite, and salicin. Mannite is uniformly attacked to a lesser degree than the other substances. Inulin is not fermented. Gelatin is not liquefied.

TABLE I.

The Non-Hemolytic Streptococci from the Skin.

Culture No.	Grouping.*	Growth in bouillon.	Litmus milk.	Production of acid in.							
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Sk 2	Pairs; chains of 4.	Turbid.	Coagulated on boiling.	2.7†	2.4	2.8	2.7	2.6	0.2	0.9	2.8
" 11	Pairs and S. C.	"	" "	3.3	2.9	2.9	3.2	2.7	0.2	1.2	3.2
" 12	" "	"	" "	3.2	2.6	3.5	3.2	2.9	0.0	2.0	3.0
" 20	" "	"	Acid.	3.1	2.6	3.2	2.7	2.9	0.2	2.5	3.2
" 23	" "	"	Coagulated on boiling.	3.2	2.9	3.3	3.0	2.8	0.1	1.8	3.1
" 24	" "	"	" "	2.9	2.5	2.9	2.8	2.3	0.1	2.5	2.7
" 26	" "	"	" "	2.4	2.4	2.2	2.2	2.4	0.0	1.0	2.4
" 27	" "	"	" "	4.3	3.3	3.3	3.6	3.1	0.0	2.5	4.0
" 29	" "	"	" "	4.2	3.6	4.1	4.3	3.5	0.0	1.2	3.3
" 30	" "	"	" "	2.6	2.1	2.4	2.6	2.5	0.0	2.9	2.4
" 32	" "	"	" "	2.7	2.2	2.6	2.4	3.0	0.0	1.3	2.4
" 34	" "	"	" "	3.6	2.9	3.3	3.1	2.8	0.0	1.4	2.7
" 35	" "	"	" "	3.0	2.7	2.6	2.9	2.7	0.0	1.0	2.7

* The length of chains has been indicated as follows: S. C., 4 to 8 cocci; M. C., chains of 8 to 16; L. C., chains of more than 20.

† Only net acid production has been recorded in the tables. The addition of 0.7 to the figures in the various columns will give a close approximation of the total acidity.

Twenty-one strains of streptococci have been isolated from the feces. Their cultural characters are shown in Table II. The bulk of fecal streptococci fall into one large group. This group is characterized by the formation of large amounts of acid in dextrose, lactose, saccharose, maltose, raffinose, inulin, and salicin. Litmus milk is firmly coagulated and litmus is usually reduced. Gelatin is not liquefied. Two cultures, Nos. F 11 and F 20, belong to the skin group.

Since the vaginal, skin, and fecal streptococci possess specific characters rendering their classification definite, each type should be readily recognized if found in milk.

TABLE II.

The Non-Hemolytic Fecal Streptococci.

Culture No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.							
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
F 4	Pairs and S. C.	Turbid.	Firmly coagulated.	7.3	5.1	6.5	6.3	6.3	7.3	0.0	5.5
" 6	" "	"	" "	8.3	4.9	7.1	7.6	7.0	6.5	0.0	5.0
" 18	" "	"	" "	7.2	5.4	5.6	7.0	5.8	7.0	0.0	5.0
" 21	" "	"	" "	6.4	4.6	5.7	5.7	5.7	6.6	0.0	5.5
" 28	" "	"	" "	6.4	5.0	5.5	5.9	5.7	6.6	0.1	5.0
" 29	" "	"	" "	5.3	4.4	5.2	4.9	5.1	6.0	0.0	4.5
" 30	" "	"	" "	5.7	4.2	5.3	5.7	5.5	5.7	0.0	5.3
" 31	" "	"	" "	6.5	6.4	5.8	5.8	5.1	6.4	0.0	6.0
" 32	" "	"	" "	6.2	4.0	6.0	5.6	6.0	6.7	0.0	5.2
" 33	" "	"	" "	7.2	4.5	5.9	5.8	5.7	6.4	0.0	5.4
" 34	" "	"	" "	5.9	4.6	5.4	5.8	5.8	6.0	0.0	5.4
" 35	" "	"	" "	5.9	4.3	5.8	5.9	6.5	6.5	0.0	5.6
" 36	" "	"	" "	6.4	4.7	5.7	6.1	4.9	6.3	0.0	5.1
" 37	" "	"	" "	6.0	4.2	6.0	5.2	5.4	6.0	0.0	4.6
" 39	" "	"	" "	6.4	4.5	5.3	4.5	6.5	6.0	0.0	5.3
" 40	" "	"	" "	6.4	4.0	5.5	5.3	6.1	6.3	0.0	5.1
" 2	" "	"	" "	7.4	5.8	5.9	6.2	3.3	0.0	0.0	5.8
" 11	" "	"	Coagulated on boiling.	3.2	2.4	3.1	3.1	2.9	0.1	1.8	3.0
" 20	" "	"	Acid.	3.9	3.0	2.7	3.1	3.0	0.1	1.4	3.5
" 16	M. C.	"	Unchanged.	4.1	0.0	4.1	4.3	3.2	2.8	3.4	4.0
" 46	Pairs and S. C.	"	Acid.	2.1	2.0	2.1	2.0	2.0	0.0	0.2	2.0

Examination of Market Milk for Streptococci.

Pint bottles of milk from the herd supply were chosen at random at various times. 1 cc. of milk was diluted in 9 cc. of sterile salt solution. After shaking vigorously, 0.5 cc. of the mixture was plated with 12 cc. of melted agar and 1 cc. of defibrinated horse blood. After incu-

bation for 24 hours at 38°C. the plates were examined. In all, twenty-six samples have been plated. The average for all the samples has been 2,850 organisms per cubic centimeter, of which 15.5 per cent have been streptococci. The proportion of streptococci varied over a wide latitude; the greatest number recorded was 45 per cent of the

TABLE III.

The Non-Hemolytic Streptococci from Milk.

Culture No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Results of agglutination with mastitis streptococcus, serum dilution 1:1,000.
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
BM 8	L. C.	Clear.	Firmly coagulated.	3.4	2.8	2.8	3.0	0.0	0.1	0.1	2.3	+*
" 13	"	"	" "	4.5	3.5	3.2	3.8	0.0	0.1	0.0	3.1	− (1:500 = ++)
" 17	"	"	" "	4.5	3.4	3.3	3.0	0.0	0.1	0.0	3.2	+++
" 27	M. C.	Turbid.	" "	4.3	3.6	3.3	3.4	0.0	0.1	0.0	3.2	+++
" 32	L. C.	"	" "	4.7	3.9	3.4	3.8	0.0	0.0	0.0	2.9	+++
" 39	"	"	" "	4.4	3.8	3.8	4.0	0.0	0.1	0.0	3.2	− (1:500 = +)
" 49	M. C.	"	" "	3.8	3.8	3.1	3.2	0.1	0.1	0.0	2.9	+++
" 53	L. C.	"	" "	4.4	3.9	3.9	4.0	0.0	0.0	0.0	3.8	+++
" 61	"	"	" "	4.5	3.6	3.5	4.1	0.1	0.1	0.1	3.8	+
" 69	S. C.	"	" "	4.6	3.7	4.4	4.2	0.0	0.1	0.0	3.7	−
" 72	M. C.	Clear.	" "	4.5	4.1	4.1	3.8	0.0	0.1	0.0	2.8	+
" 74	L. C.	"	" "	4.6	3.5	3.2	4.0	0.0	0.0	0.0	3.3	++
" 78	M. C.	Turbid.	" "	4.6	4.0	3.3	3.8	0.0	0.0	0.0	3.5	++
" 89	L. C.	"	" "	4.3	3.5	3.5	4.0	0.0	0.0	0.0	2.9	+++
" 92	M. C.	"	" "	4.3	3.5	3.3	3.5	0.0	0.0	0.0	3.3	+++
" 23	S. C.	"	Coagulated on boiling.	3.0	2.9	3.1	2.7	2.8	0.0	1.0	3.1	−

* +++ indicates complete agglutination; ++, marked clumping without entire clearing of the fluid; +, moderate agglutination. A negative reaction is noted as −.

total number of organisms. Of the 72 streptococci isolated, 56 are of the hemolytic type. The others are non-hemolytic. These figures indicate a greater proportion of hemolytic streptococci, although this is not necessarily the case. The hemolytic colonies are more

easily differentiated from the others and many of the deeper non-hemolytic colonies may have been overlooked. In Tables III and IV the cultural characters of the milk streptococci are given.

It will be observed that the non-hemolytic streptococci with one exception fall into the mastitis group. They all produce considerable acid in dextrose, lactose, saccharose, maltose, and salicin. Milk is firmly coagulated but litmus is not reduced. In addition, all but one agglutinate with their specific group serum. This serum was obtained by the immunization of a cow with a strain of non-hemolytic mastitis streptococcus. Strain BM 23 agrees in neither its cultural nor immunological characters with the other organisms; it is probably from the skin, since it ferments dextrose, lactose, saccharose, maltose, raffinose, mannite, and salicin.

Of 56 strains of hemolytic streptococci, 43 agree in their cultural characters and agglutination affinities with the hemolytic types most frequently associated with bovine mastitis. Like the non-hemolytic group, they produce considerable acid in dextrose, lactose, saccharose, and maltose. Salicin may or may not be fermented. Milk is firmly coagulated without the reduction of litmus. These high acid-producing strains (both hemolytic and non-hemolytic) occurred in the milk in large numbers. They make up the bulk of the streptococcic flora.

The cultural characters of the other thirteen strains differ from those associated with mastitis. They ferment dextrose, lactose, saccharose, and maltose, but none has attacked inulin, raffinose, mannite, or salicin. One culture fails to attack saccharose. Milk is coagulated but not firmly. The curd is flocculent. Litmus is often partially reduced. The amount of acid produced in dextrose is considerably lower than that recorded for the mastitis type. In addition, the hydrogen ion concentration is higher, pH 5.0 to 5.3. Gelatin and coagulated serum are not liquefied. An additional point of differentiation between members of this group and those of the mastitis group is the difficulty with which they are suspended in 1 per cent sodium chloride solution. The mastitis strains all readily enter into suspension. The others sediment spontaneously within 3 or 4 hours. When freshly isolated many of these strains grow with difficulty in fermented or plain bouillon, even when carbohydrate is added; the addition of 5 to 10 per cent of sterile horse serum to the media insures

TABLE IV.

The Hemolytic Streptococci from Milk.

Culture No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Results of agglutination test with mastitis streptococcus, serum dilution 1:200.	Hydrogen ion concentration in dextrose, fermented broth.
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.		
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*pH*
BM 1	M. C.	Turbid.	Firmly coagulated.	4.7	3.8	3.9	4.3	0.0	0.0	0.0	0.1	+++	
" 5	"	"	" "	4.7	3.8	4.0	4.3	0.1	0.1	0.1	0.1	+++	4.4
" 6	"	"	" "	4.5	3.9	3.8	4.0	0.1	0.1	0.0	0.0	+++	4.5
" 9	S. C.	"	" "	4.2	3.8	3.3	3.5	0.1	0.0	0.0	0.0	+++	4.7
" 10	M. C.	"	" "	3.9	3.6	3.9	4.0	0.0	0.0	0.0	0.0	++	4.7
" 11	"	"	" "	4.6	3.9	3.5	4.0	0.1	0.1	0.0	0.0	+++	4.5
" 14	"	"	" "	5.0	4.1	4.0	4.1	0.1	0.0	0.0	0.0	+++	
" 15	L. C.	"	" "	4.7	4.0	4.1	3.6	0.0	0.1	0.0	0.0	+++	
" 16	"	"	" "	4.4	3.8	4.0	4.2	0.1	0.1	0.0	0.0	++	
" 18	"	"	" "	4.8	3.7	3.9	4.2	0.1	0.0	0.0	0.1	+++	
" 19	"	"	" "	4.0	3.7	3.8	3.9	0.2	0.2	0.1	0.1	+++	
" 20	M. C.	"	" "	4.6	3.7	3.9	4.0	0.0	0.1	0.0	0.1	+	4.5
" 21	L. C.	"	" "	4.9	3.8	3.9	4.3	0.0	0.0	0.1	0.1	+++	
" 25	"	Clear.	" "	4.1	3.2	3.5	3.5	0.0	0.1	0.0	0.0	+++	
" 33	M. C.	Turbid.	" "	4.0	3.8	3.6	3.6	0.0	0.1	0.0	0.0	+++	
" 35	"	"	" "	4.9	4.0	3.9	4.2	0.0	0.0	0.0	0.1	++	
" 37	L. C.	"	" "	5.4	3.8	3.8	4.6	0.0	0.0	0.0	0.0	+++	4.6
" 40	S. C.	"	" "	4.6	3.9	4.0	4.3	0.1	0.0	0.1	0.0	+++	
" 42	M. C.	"	" "	4.5	4.0	3.9	4.0	0.0	0.0	0.0	0.0	+++	
" 43	"	"	" "	5.3	4.4	4.4	4.8	0.0	0.0	0.1	0.0	+	4.4
" 45	"	"	" "	4.9	4.0	4.0	4.0	0.0	0.0	0.0	0.0	+++	
" 46	S. C.	"	" "	4.8	4.0	4.0	4.1	0.0	0.0	0.0	0.0	++	
" 47	L. C.	"	" "	4.5	3.9	3.9	4.3	0.0	0.0	0.0	0.0	+++	

BM 51	M. C.	Turbid.	Firmly coagulated.		4.7	3.8	3.9	4.5	0.0	0.0	0.0	0.0	+++	
" 52	"	"	"	"	4.7	3.9	4.1	4.4	0.0	0.0	0.1	0.0	+++	
" 54	L. C.	"	"	"	4.6	4.1	4.1	4.5	0.0	0.1	0.0	0.1	+++	
" 56	"	"	"	"	4.7	4.1	3.8	4.1	0.0	0.0	0.0	0.1	+++	
" 57	S. C.	"	"	"	4.0	3.8	3.8	4.1	0.0	0.1	0.0	0.0	+++	
" 59	L. C.	"	"	"	4.5	4.0	3.8	4.1	0.0	0.0	0.0	0.0	+++	
" 62	M. C.	"	"	"	4.6	4.1	4.1	4.5	0.1	0.0	0.1	0.1	+++	
" 66	L. C.	"	"	"	4.5	4.1	4.1	4.2	0.0	0.1	0.1	0.0	+++	
" 70	M. C.	"	"	"	4.8	4.0	4.1	4.4	0.1	0.0	0.0	0.0	++	
" 73	"	"	"	"	4.3	3.4	4.0	3.8	0.0	0.0	0.1	0.1	+++	
" 76	S. C.	"	"	"	4.6	4.1	4.1	4.1	0.0	0.0	0.0	0.0	+++	
" 81	M. C.	"	"	"	4.9	4.0	3.8	5.0	0.0	0.0	0.0	0.0	+++	
" 85	"	"	"	"	5.0	3.9	3.8	4.4	0.0	0.0	0.0	0.0	+++	
" 87	L. C.	"	"	"	5.0	3.7	3.7	4.0	0.0	0.0	0.0	0.0	+++	
" 90	"	Clear.	"	"	4.8	4.3	4.0	3.9	0.0	0.1	0.0	0.0	+++	
" 2	"	"	"	"	4.2	3.9	4.0	3.8	0.1	0.0	0.1	2.5	+++	4.7
" 4	"	"	"	"	4.4	3.9	3.9	4.1	0.1	0.0	0.1	3.1	+++	4.5
" 24	"	Turbid.	"	"	4.7	3.9	4.3	4.0	0.1	0.0	0.0	2.4	+++	
" 29	"	"	"	"	4.9	4.6	4.1	4.2	0.0	0.0	0.0	3.2	+++	
" 63	"	"	"	"	4.4	4.3	4.5	4.0	0.1	0.0	0.0	2.5	+++	
" 17A	"	Clear.	Coagulated on boiling.		3.4	3.0	2.6	3.3	0.0	0.0	0.0	0.0	Spontaneous precipitation.	5.2
" 22	S. C.	Turbid.	Firmly coagulated.		3.3	3.0	2.5	3.3	0.1	0.0	0.0	0.0	" "	5.1
" 30	M. C.	Clear.	Coagulated, not firmly.		3.6	2.7	1.1	3.5	0.0	0.0	0.0	0.0	" "	5.2
" 44	"	Turbid.	"	" "	3.3	2.7	2.6	3.3	0.0	0.1	0.0	0.0	—	5.2
" 58	S. C.	"	"	" "	3.4	3.4	2.7	2.8	0.0	0.1	0.0	0.0	Spontaneous precipitation.	5.2
" 60	"	"	"	" "	3.4	3.0	1.8	3.1	0.0	0.1	0.0	0.0	" "	5.0
" 64	L. C.	Clear.	"	" "	3.1	3.0	2.4	3.0	0.0	0.0	0.0	0.0	" "	5.2
" 67	"	"	"	" "	3.3	3.1	1.2	3.0	0.0	0.0	0.0	0.0	" "	5.3
" 77	M. C.	Turbid.	"	" "	3.5	2.9	2.9	3.7	0.0	0.0	0.0	0.0	" "	5.2
" 82	S. C.	"	"	" "	3.5	3.1	2.8	3.6	0.0	0.0	0.0	0.0	" "	5.2
" 84	"	"	"	" "	3.6	2.9	2.8	3.6	0.0	0.0	0.0	0.0	" "	5.0
" 88	M. C.	"	"	" "	3.0	2.9	2.6	3.5	0.0	0.0	0.1	0.0	" "	5.2
" 71	"	"	"	" "	3.3	3.1	0.0	3.3	0.0	0.0	0.0	0.1	" "	5.2

moderate growth. Growth, however, takes place slowly. In a number of instances several attempts were made before fermentation was obtained in saccharose. The colonies in blood agar plates are smaller than those produced by the members of the larger group. The surface colonies rarely reach 1 mm. in diameter. They are sharply raised and surrounded by a clear zone of hemolysis. The deep colonies are exceedingly small, biconvex or ovoid in shape, and produce clear hemolytic areas 1.5 to 4 mm. in diameter after 24 hours incubation. After 48 hours the diameter of the hemolytic area is frequently doubled or trebled.

Considerable differences in the morphology have been observed. Chains of 6 to 15 elements are usually observed in liquid media. The individual elements are usually round, slightly elongated, and even rod-shaped. In the water of condensation of blood agar cultures the organisms appear as diplococci and short rods. Clubbed ends are frequently observed. All forms retain the stain by Gram's method. The morphology of strains grown on artificial media for some time becomes more fixed, since the organisms grow in bouillon as typical short chained streptococci.

Five of the cultures from this group chosen at random were tested for pathogenicity in rabbits. 1 cc. of a 24 hour serum bouillon culture was injected into the ear veins. The animals were not appreciably affected. Their temperatures ranged well within the normal limits. Two of the rabbits were killed 15 days after injection. Necropsy failed to reveal localization either in the heart valves, the joints, or the viscera. Eight other cultures in 0.1 cc. doses were injected into the peritoneal cavities of white mice. The mice remained well.

Members of this group appeared in half the samples of milk examined, but in very small numbers; usually only one, two, or three colonies developed in plate cultures.

The low acid-producing strains in market milk had not been found in any of the regions examined. In previous studies such organisms had not been noted in plates made directly from udder milk. It was determined to trace their source back from the bottled milk. On this particular dairy farm it is customary to collect the milk from individual cows into 40 quart cans. These cans are removed from the barns, and sent to the creamery as soon as they are filled. The con-

tents of the cans, on the average, are made up of the mixed milk of five or six cows. Samples were obtained from the various cans from each barn. Characteristic colonies of the low acid-producing streptococci were observed in certain samples of can milk. This observation pointed to one of three sources of entrance: udder infection, exfoliations from the skin of the udder, or contamination by the milker during milking. Accidental contamination from exfoliations from the skin or from the milker did not seem probable, especially as covered milk pails are used. The skin of the udder of 50 cows was examined with negative results. It was determined to examine the udder milk from cows whose mixed milk was known to contain streptococci of the type sought. The search proved more difficult than at first supposed. Many udders were found infected with the typical bovine types, but comparatively few harbored the low acid producers. Of 50 cows in one barn the milk from two revealed organisms of this type. Usually, however, one finds at least one in 50 harboring the low acid-producing streptococci. The number of colonies which develop from 1 cc. of milk is usually small (40 to 80). In one instance, however, plates from the milk revealed 900 colonies per cubic centimeter. Undoubtedly more udders are invaded than indicated, since these streptococci are exceedingly delicate and are crowded out in the plates by the ordinary udder streptococci and other organisms. Higher dilutions in milk with high counts entirely eliminate them from the plate cultures since they occur only in small numbers.

Another streptococcus similar in many respects to the low acid-producing strains was found in milk drawn directly from the udder. It, however, ferments mannite and salicin in addition to dextrose, lactose, saccharose, and maltose. Such strains are also low acid producers and grow poorly in plain or fermented bouillon. The colonies and hemolytic characters in blood agar plates are indistinguishable from those noted for the low acid-producing streptococci.

DISCUSSION.

The predominating types of streptococci in the market milk are those of mastitis. It is true that fecal and skin streptococci may gain access to the milk, but they are practically absent in fresh milk

if it is kept under proper conditions. It is conceded, however, that certain of these streptococci may play an important part in the souring of milk; as for instance, the high acid-producing fecal strains.

Heinemann[5] in considering the significance of streptococci in milk came to the conclusion that *Streptococcus lacticus*, supposedly the most common type of milk streptococci, agrees in its morphological and cultural characters with pathogenic, fecal, and sewage streptococci. Miller[6] reached the same conclusion. He asserted that *Streptococcus pyogenes* and *Streptococcus lacticus* are indistinguishable by present methods of study. Rogers and Dahlberg reached the same conclusion in regard to certain udder streptococci.

The most frequent types of streptococci which I have observed are identical with those causing mastitis. It has been shown repeatedly that such streptococci are found in the udder before clinical manifestations of the disease appear and may persist for long periods after symptoms have subsided. That such streptococci have been isolated from market milk is shown by Davis' protocols. The only point of difference between his milk streptococci and a strain from a case of mastitis was in the virulence of the latter for rabbits. The writer has never isolated strains of mastitis streptococci of purely bovine origin which possessed high pathogenic properties for rabbits. The question is raised whether or not many belonging to the so called *Streptococcus lacticus* group may not be identical with mastitis streptococci.

The experiments of Mathers[7] have a distinct bearing on this point. He injected human, mastitis, and milk streptococci into the udders of cows. The human and mastitis strains produced a severe mastitis which became chronic. The non-hemolytic *Streptococcus lacticus* gave rise to an acute mastitis which displayed all the symptoms observed in spontaneous mammitis (swelling, heat, pain, and purulent milk). The injection of a culture of a hemolytic streptococcus from milk gave much the same result. The inflammation resulting from these injections disappeared within 2 weeks. The fact remains, however, that both supposedly non-pathogenic cultures produced disease. It is admitted that the inflammation is of short duration, but such is not infrequent in certain cases of spontaneous infection.

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[5] Heinemann, P. G., *J. Infect. Dis.*, 1906, iii, 173.

[6] Miller, W. W., *Bull. Hyg. Lab., U. S. P. H., No. 41*, 1908, 479.

[7] Mathers, G., *J. Infect. Dis.*, 1916, xix, 222.

It is difficult to harmonize the results obtained by Savage[8] in England with those obtained by me. The method of handling and caring for cows, the precautions taken to avoid contamination of the milk, etc., make such comparisons impossible. The types of streptococci producing mastitis in the district in which Savage's observations were made differ to a considerable degree from those which I have isolated. However, he brings out the point that most of the streptococci which he found in milk are of udder origin, and considers that the udder group is intermediate between the fecal and mastitis streptococci.

The group of low acid-producing hemolytic streptococci is interesting. Their hydrogen ion concentration readings are significant. According to Avery and Cullen's[9] observations, they fall well within the human group. In fact, in Table VIII of their paper the readings of three strains (M. 53, M. 86, and J. 1), isolated by the writer, are given. These strains were obtained from milk. At that time it was stated that they were not associated with mastitis, but might have been human contaminations, or arisen from the skin or feces of the cow. The latter postulation has been disproved, since hemolytic streptococci have not been found on the skin or in the feces of the cows supplying milk in this herd. These strains produce about the same amount of titratable acid in dextrose as the human streptococci. They differ from pathogenic human streptococci in character of the colonies produced on blood agar and in their ability to coagulate milk. They possess no virulence for mice and rabbits. It is possible, however, that such streptococci may be of human origin. Holman[10] designates streptococci which are hemolytic and ferment lactose but do not attack mannite, inulin, or salicin as *Streptococcus anginosus* (Andrewes and Horder). Several have reported the presence of such organisms in the human nose, throat, and tonsils. Perhaps these organisms gain access to the udder from the milker. At any rate they do not possess severe invasive characters for cattle, since their numbers are held in check in the udder. They differ from the non-

[8] Savage, W. G., *Rep. Med. Off., 1906–07, Local Gov. Bd., Appendix B, No. 4*, 1908, 3.

[9] Avery, O. T., and Cullen, G. E., *J. Exp. Med.*, 1919, xxix, 215.

[10] Holman, W. L., *J. Med. Research*, 1916, xxxiv, 377.

salicin-fermenting mastitis streptococci both in their hemolytic properties and in their inability to produce as much acid.

On morphological grounds they resemble diphtheroids, but have been placed with the streptococci for several reasons. In addition to forming chains in bouillon, they produce the characteristic hemolytic areas associated with hemolytic streptococci. Their fermentative characters resemble those of streptococci. In cultures that have grown on artificial media for considerable periods they produce in bouillon only chains of cocci.

Both the mastitis and the other group of streptococci have been observed in the market milk from this farm during the past 2 years. During this time diseases traceable to this milk supply have not been reported. This evidence points to the low pathogenicity of either group for consumers of milk. Either species may be eliminated as a source of severe epidemics of milk-borne sore throat.

SUMMARY.

The principal source of streptococci in milk is the cow's udder. The udder streptococci fall into two broad groups; those of the larger group agree in cultural characters and agglutination affinities with mastitis streptococci; the smaller group is composed of low acid-producing streptococci. The streptococci of the latter group produce clear zones of hemolysis about surface and deep colonies in horse blood agar plates. They attack dextrose, lactose, saccharose, and maltose, but do not ferment raffinose, inulin, mannite, or salicin. Acid production in dextrose by the members of this group is about the same as that produced by human streptococci under the same conditions. The limiting hydrogen ion concentration for these pleomorphic udder streptococci in dextrose serum bouillon is within the limits of the limiting hydrogen ion concentration observed by Avery and Cullen for human streptococci.

All the streptococci from the vagina, saliva, skin, and feces have been non-hemolytic. Those from the saliva form a heterogeneous aggregation in which individuals fermenting raffinose, inulin, and mannite predominate. From the skin a characteristic streptococcus has been found. It produces acid in dextrose, lactose, saccharose, mal-

tose, raffinose, mannite, and salicin, but fails to acidulate media containing inulin. The fecal streptococci are characterized by the formation of large amounts of acid in dextrose, lactose, saccharose, maltose, raffinose, inulin, and salicin. Mannite is not fermented. Neither the fecal nor the skin streptococci have been isolated from the bottled milk with any great frequency.

EXPERIMENTAL STUDIES ON DIABETES.

Series I. Production and Control of Diabetes in the Dog.

1. Gross Anatomic Relations of the Pancreas and Diabetes.

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The basis of the present studies has been a form of diabetes produced by removal of the greater part of the pancreas of animals, leaving a remnant about the duct secreting normally into the duodenum, thus avoiding the rapidly fatal cachexia of total pancreatectomy and also the pancreatic sclerosis and deficient digestion of Sandmeyer diabetes and affording a very close and satisfactory reproduction of the clinical disorder.[1] Because of the importance of relations between pancreas mass and body mass, and between the total pancreas and the size of remnant with which diabetes occurs, a summary will first be given of these anatomic facts in dogs, taken from the records of all the experiments suitable for this purpose.

Accuracy of Estimation of the Size of the Pancreas Remnant.

The method of operation and estimation of the remnant has been previously described.[2] In brief, a piece of the removed tissue is trimmed to imitate the remnant left *in situ;* this piece and the total tissue removed are weighed, and the absolute and relative weights of the remnant and total pancreas are thus reckoned. It is important to combine sight and touch to obtain the greatest accuracy, for feeling the comparative thickness and mass often corrects mistakes of the eye. Though practice is important, the range of error was practically constant throughout this series, and differences depended

[1] All operations were performed under ether anesthesia.

[2] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913, Chapter X.

not so much upon care, skill, or chance as upon differences in the shape of the pancreas. When, as frequently, a piece can be cut from directly beside the remnant, of practically the same shape and consistency, the agreement is generally close; but when a piece of different form and structure must be used, the error is likely to be greater.

As shown in Table I, 68 dogs freshly dead from natural or experimental causes were subjected to the usual operation and the actual remnant was immediately removed and compared with the estimated one. The total of the pancreas weights of the 68 dogs was 1,812.8 gm. The sum total of the estimated remnants was 197.5 gm., of the actual

TABLE I.

Accuracy of Estimation of the Size of the Pancreas Remnant.

Material.	No. of animals.	Combined weight of.			Maximum individual error.		
		Total pancreas.	Estimated remnants.	Actual remnants.	Absolute.	Relative, as fractions of total pancreas.	
						Estimated remnant.	Actual remnant.
		gm.	*gm.*	*gm.*	*gm.*		
Dead dogs	68	1,812.8	197.5	196.8	1.1	$\frac{1}{14}$	$\frac{1}{11} - \frac{1}{12}$
" puppies	12	74.0	9.1	9.2	0.4	$\frac{1}{9} - \frac{1}{10}$	$\frac{1}{7} - \frac{1}{8}$
Total pancreatectomies	18	419.7	45.2	43.4	0.8	$\frac{1}{8}$	$\frac{1}{10}$

remnants 196.8 gm. The greatest individual error occurred when the total pancreas weighed 70.7 gm., the estimated remnant 5.1 gm., and the actual remnant 6.2 gm. Thus, the absolute difference between the remnants was 1.1 gm., and when expressed as usual in the form of fractions, the estimated remnant was approximately $\frac{1}{14}$ and the actual remnant between $\frac{1}{11}$ and $\frac{1}{12}$ of the total pancreas. The absolute error is naturally apt to be greatest when, as here, the dog, pancreas, and remnants are large; but only rarely is the relative error sufficient to change the denominator of the fraction by more than one digit. In this series of 68 dogs, the estimated and actual remnants were equal or within 0.1 gm. of each other in 25 instances. In the other 43 animals, the estimated remnant weighed more than

the actual remnant in 22 cases, and less in 21. The difference between the estimated and actual remnants was 0.2 gm. in 17 instances, 0.3 gm. in 9 instances, 0.4 gm. in 6 instances, 0.5 gm. in 4 instances, 0.6 gm. in 2 instances, 0.7 gm. in 1 instance, 0.8 gm. in 1 instance, 0.9 gm. in 2 instances, and 1.1 gm. in the extreme instance mentioned.

A similar procedure was carried out upon twelve dead puppies, ranging in age from 2 weeks to 10 months. As the animals and organs were smaller, the absolute error was less, but the relative error was about the same. It was greatest in the largest pup, which was 10 months old, and in which the pancreas weighed 13.8 gm., the estimated remnant 1.4 gm., and the actual remnant 1.8 gm. In every other instance the agreement was within 0.1 gm.

The conditions of operation upon living animals were fully reproduced when partial pancreatectomy was performed in the course of total pancreatectomy upon eighteen dogs. The general summary is shown in Table I. The estimated and actual remnants were equal or within 0.1 gm. of each other in 4 instances. They differed by 0.2 gm. in 3 instances, by 0.3 gm. in 3 instances, by 0.4 gm. in 2 instances, by 0.6 gm. in 5 instances, and by 0.8 gm. in 1 instance. This maximum error occurred when the total pancreas weighed 31.4 gm., the estimated remnant 3.9 gm., $= \frac{1}{8}$, and the actual remnant 3.1 gm., $= \frac{1}{10}$ of the total pancreas.

It may be concluded from these figures that the method of estimation is exact enough to be used for statistical studies. But close as is the agreement on the average and in the majority of cases, there is a large minority of errors of a magnitude sufficient to determine the occurrence or absence of diabetes. Accordingly, if the influence of any agencies in causing or preventing diabetes is to be tested with the aid of this operative method, these possible limits of error must be considered in judging the results.

Body Weight, Pancreas Weight, and the Tendency to Diabetes.

If the operative estimates are accepted as accurate to the degree above shown, a series of dogs subjected to partial pancreatectomy is available for analysis (Table II).

TABLE II.

Analysis of Results of Partial Pancreatectomies.

No. of dogs.	Body weight.	Pancreas weight.			Weight of pancreas per kilo of body weight.			Maximum fraction of pancreas with which diabetes occurred.
		Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	
	kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
1	2	9.0			4.50			
2	3	9.9	10.0	9.7	3.30	3.33	3.23	
8	4	12.1	16.8	8.9	3.02	4.20	2.22	$\frac{1}{7}-\frac{1}{8}$
18	5	14.2	20.5	8.7	2.84	4.10	1.74	$\frac{1}{8}$
20	6	15.5	18.2	11.2	2.59	3.03	1.87	$\frac{1}{7}$
23	7	17.5	29.4	10.9	2.50	4.20	1.56	$\frac{1}{7}$
26	8	19.7	28.3	12.6	2.46	3.54	1.58	$\frac{1}{6}-\frac{1}{7}$
33	9	22.0	31.1	16.5	2.44	3.45	1.83	$\frac{1}{10}$
35	10	22.3	31.3	14.0	2.23	3.13	1.40	$\frac{1}{7}$
25	11	25.5	38.9	17.3	2.32	3.54	1.57	$\frac{1}{8}-\frac{1}{9}$
36	12	25.7	42.5	15.5	2.14	3.54	1.30	$\frac{1}{8}$
27	13	27.8	39.4	18.7	2.14	3.03	1.44	$\frac{1}{6}$
23	14	30.2	44.1	20.5	2.16	3.15	1.46	$\frac{1}{9}$
34	15	31.4	50.3	20.6	2.09	3.35	1.37	$\frac{1}{7}$
22	16	35.9	63.4	27.3	2.24	3.96	1.71	$\frac{1}{8}$
13	17	33.7	50.3	26.6	1.98	2.96	1.56	$\frac{1}{4}-\frac{1}{5}$
27	18	36.5	49.6	23.1	2.03	2.76	1.28	$\frac{1}{5}$
13	19	34.8	52.9	28.1	1.83	2.78	1.48	$\frac{1}{5}$
11	20	35.5	46.1	23.5	1.77	2.30	1.17	$\frac{1}{7}$
7	21	41.5	54.5	29.1	1.98	2.60	1.39	
2	22	35.1	38.5	31.8	1.59	1.75	1.45	$\frac{1}{6}-\frac{1}{7}$
6	23	46.5	54.7	38.3	2.02	2.38	1.66	$\frac{1}{5}$
5	24	45.0	51.9	39.4	1.87	2.16	1.64	$\frac{1}{7}$
2	25	66.3	94.4	38.0	2.65	3.77	1.52	
7	26	54.2	67.5	43.5	2.08	2.60	1.67	$\frac{1}{5}-\frac{1}{10}$
1	27	51.1			1.89			
1	28	40.8			1.46			
2	29	73.8	95.3	52.0	2.54	3.28	1.79	
1	30	35.9			1.19			
1	31	70.7			2.28			
2	34	72.8	88.9	56.8	2.14	2.62	1.67	
1	36	58.9			1.63			$\frac{1}{6}$
1	37	82.8			2.24			
1	42	81.8			1.95			

The weight of the pancreas ranged from a minimum of 1.17 gm. to a maximum of 4.5 gm. per kilo of body weight. These figures are derived from animals which were chosen as apparently normal in all

respects, free from any disturbing factors such as notable obesity or emaciation, extreme age or youth, pancreatic or other disease, etc., and in which, moreover, the normality of the pancreatic tissue was in a high proportion of instances confirmed by microscopic examination. They are therefore of statistical value from the pure anatomic standpoint, and also as a basis of comparison for some of the ensuing studies.

A detailed record was kept of the sex, apparent age, breed, color and character of coat, and other characteristics of all animals. Analysis indicates (*a*) that sex is immaterial, and also (*b*) that in adult animals variations of age are without perceptible influence in regard to the pancreas weight. (*c*) No breeds were found with characteristically large or small pancreas. (*d*) On the other hand, the numerous crosses and admixtures of different breeds were suggestive. Anyone handling large numbers of dogs will observe that while characters are generally blended, the size and form of organs are among those which are sometimes transmitted almost like Mendelian units. Thus, the large square bulldog head with its undershot jaw may be found on a fox-terrier body, and examples of obvious bastardy in legs, tail, coat, and other features are too common to mention. The uncertain ancestry of all the animals and the mixture in the majority make tabulation or exact conclusions impossible for both (*c*) and (*d*). But there is no doubt that the variations in size due to cross-breeding pertain also to visceral organs. One of the clearest examples is the occasional finding of the deep thorax and large lungs of a greyhound in a mongrel with the other characters of some other breed or mixture. The impression was gained that in the crossing of large and small dogs, the size of the pancreas in the offspring is usually a blend roughly proportioned to the body weight, but in some instances it is chiefly derived from either the large or the small parent, so that the ratio of pancreas weight to body weight is altered, and the animal has an exceptionally large or small pancreas in proportion to the size of its body.

Dogs offer unique advantages for tracing a range of relations between body weight and pancreas weight. No other mammal exhibits such a gamut of sizes as here represented between the 2 and 42 kilo weights for normal adults of the same species. In a broad and

general way, with allowance for the exceptions mentioned in the preceding paragraph, the table indicates a lowering of the proportional size of the pancreas as the body weight rises. The rule in this general sense applies to all three columns, of average, maximum, and minimum weights. In other words, small dogs generally possess more pancreas tissue in proportion to their body mass than large dogs. According to well known laws, the basal metabolism of small dogs per kilo is higher than that of large dogs, and with allowances for activity, warmth of coat, and other modifying factors, their actual daily metabolism in proportion to weight is doubtless higher. As one of the principal studies of the entire investigation pertained to the relation of the pancreatic function respectively to the body mass and to the metabolism, it was interesting to observe that dogs seem normally to be provided with pancreatic tissue rather in proportion to their metabolism than to their body mass.

The observations were also directed to determine whether a uniform mathematical law can be established for the occurrence of diabetes whenever a fixed proportion of the pancreas is removed, or whether general or individual exceptions exist. With regard to the statements in the preceding paragraph, it might be inquired whether small dogs have a larger endowment of pancreas corresponding to larger needs, or merely a greater margin of safety. It is known from veterinary literature that dogs are occasionally subject to spontaneous diabetes. In human pathology, a tendency to diabetes has sometimes been attributed either to small size of the pancreas in gross, or to deficiency of islands. In view of the impracticability of any extensive counting of islands, there was a question whether dogs having a small pancreas would show a corresponding susceptibility to diabetes, or whether differences in the functional capacity of equal masses of pancreatic tissue would be demonstrable, due to variations in island content or other differences. Dogs exhibit contrasts of nervous and phlegmatic temperaments almost as extreme as those of men. Also some varieties of dogs have resulted from close inbreeding, and special characters have sometimes been cultivated at the price of constitutional vigor. As shown in Table II, wide differences were observed in the size of the pancreas remnant with which diabetes occurred; but these were rare and did

not conform to any of the causes above considered. Special attention was paid to the few instances in which diabetes occurred with $\frac{1}{6}$ to $\frac{1}{4}$ of the pancreas present, but only after several years was it demonstrated that the explanation lay in inflammatory changes, as described in a paper to be published later. For the most part, large dogs develop diabetes more readily and are more satisfactory for the purpose than small ones, but accessory factors enter in. The small pancreas remnant in a small dog is traumatized in operation to a relatively greater extent than the larger remnant of a large dog. This difference is overbalanced by the greater vigor and resistance of large dogs. The small ones are more subject to loss of appetite, cachexia, and distemper, which interfere with the development of active diabetes. Owing to the modifying influences, no uniform rule can be deduced from the last column of Table II. The present figures, covering a larger number of animals, confirm previous findings[2] that, with few exceptions, mild diabetes occurs when the remnant is $\frac{1}{8}$ to $\frac{1}{9}$ of the pancreas, and severe diabetes when the remnant is about $\frac{1}{10}$ of the pancreas. The internal secretory capacity of equivalent fractions of pancreatic tissue is uniform as far as these observations could determine, with no sign of variations due to gross size of the organs, differing island content, nervous control, or constitutional vigor or degeneracy. Except for inflammatory changes as mentioned, no special tendency to diabetes was evident in any dog of the series.

The observations outline the limits of error under this procedure. A demonstration of the influence of any agency in producing or preventing diabetes, by a comparison of test animals and controls, must rest upon uniform results in a considerable series, and the differences in question must generally amount to several grams of pancreas tissue. Other experiments have shown that a partially depancreatized animal, which has merely a lowered tolerance and cannot be made diabetic by any quantity or duration of feeding, may be so close to the verge that diabetes results from the removal of only a fraction of a gram of additional pancreatic tissue. Also definite variations in the severity of diabetes may be produced, for

example by overfeeding. The most delicate method, therefore, consists in tests upon the same rather than upon different animals. Due attention must always be paid to accidental modifying factors, especially body weight, pancreatitis, and cachexia.

Hypertrophy of the Pancreas Remnant.

The former series of experiments contained several examples of great hypertrophy of the pancreas remnant. Thus, in Dog 104[3] the pancreatic tissue left at the original operation on Oct. 1 was estimated at less than 6 gm. On Nov. 27, 6 gm. of tissue were removed, and the remnant found at autopsy on Dec. 23 weighed 8.1 gm. Also[4] in Dog 151 the remnant estimated at operation on Nov. 21 was 3.2 gm., while that found at autopsy on Dec. 4 was 11.3 gm.; and in Dog 152 the remnant estimated at operation on Nov. 24 was 4.4 gm., while that found at operation on Dec. 5 was 10.4 gm. In Dog 125[5] the remnant estimated at operation on Oct. 26 was 2.4 gm., and that found at autopsy on Nov. 8 was 7.7 gm. In Dog 148[6] the remnant estimated at operation on Nov. 16 was 3.3 gm., and that found at autopsy on Dec. 19 was 13.3 gm.

Hypertrophy to such an extreme degree is highly exceptional. Tables were compiled from a series of dogs which had undergone removal of most of the pancreas in single operations and were free from any known disturbing influence. The data concerning alterations in weight of the pancreas remnant are summarized in Table III.

If $\pm$ 0.4 gm. is accepted as a rough estimate of the possible limits of error, it is seen that in 35 instances the weight of the remnant remained practically stationary. The 17 cases of atrophy represented essentially traumatic fibrosis. Hypertrophy occurred in the majority of cases in the total series, but was generally slight, so that in only 12 instances was the remnant found more than doubled in size. To save reproduction of extensive detailed data, the following points may be noted as developed by analysis.

Occurrence.—Changes occur in the size of the pancreas remnant beyond any possible error of estimation. In calculations of the proportion of pancreas removed, the operative estimate should be used in preference to the weight of tissue at autopsy.

[3] Allen,[2] pp. 485–486.
[4] Allen,[2] p. 490.
[5] Allen,[2] p. 491.
[6] Allen,[2] p. 959.

State of Nutrition.—Precautions were taken to make sure that the changes in weight did not represent mere differences in fullness and emptiness of the acini. The series also included changes of the nutritive state in both directions. Gain of body weight did not necessarily correspond to increase in the pancreas remnant. Also, though the general wasting in emaciation affects the pancreas, the hypertrophy was all the more evident because some of the most marked examples were found in animals which lost a large proportion of their body weight between operation and autopsy.

Character of Tissue.—In certain instances, especially soon after operation, the increase of weight may be due to inflammatory tissue,

TABLE III.

Alterations in the Weight of the Pancreas Remnant after Partial Pancreatectomy.

No. of instances in which weight of pancreas remnant at autopsy was found within ± 0.4 gm. of estimate at operation	35
No. of instances of atrophy beyond the above limit of error	17
Average atrophy of the group (in percentage of estimated weight of remnant)	27.4 per cent.
Maximum atrophy	45 " "
No. of instances of hypertrophy to less than 150 per cent of the estimated weight	28
" " " " " " 150 per cent of the estimated weight	30
" " " " " " 200 " " " " " "	30
" " " " " " 300 " " " " " "	8
" " " " " " 400 " " " " " "	4

but this is not the true hypertrophy referred to. Sometimes also fairly normal parenchyma may be distorted or more or less encapsulated by superficial scar tissue. But in the best examples the remnant is free from perceptible sclerosis and consists of lobulated parenchyma normal in appearance and consistency. Microscopic examination confirms the absence of fibrosis. The acini may be particularly large and crammed with secretion. As described for Dogs 148 and 151[7] islands may be scanty as if the hyperplasia had been limited chiefly to the acinar tissue, or fairly abundant, as if the islands had kept pace with the proliferation of the other structures.

[7] Allen,[2] pp. 761, 969.

Size of Remnants.—The absolute size of the remnant is not a determining factor in hypertrophy. The remnants in Dogs 104, 125, 148, 151, and 152, mentioned above, were of various sizes. The later series includes remnants of 8 to 12 gm. which increased to 14 to 22 gm., also a remnant of 0.8 gm. which increased to 2 gm., one of 1.6 gm. which increased to 5.4 gm., and one of 1.8 gm. which increased to 6.5 gm. Correspondingly, the relative size of the remnants did not govern hypertrophy. Some of the larger remnants referred to were as much as $\frac{1}{4}$ of the pancreas, while the 0.8 gm. remnant mentioned was only $\frac{1}{18}$ of the total organ.

Relation to Diabetes.—It follows from the last statement that diabetes was not a determining factor in hypertrophy, which was present or absent in diabetic and non-diabetic animals indiscriminately. In other words, the stimulus did not apparently originate from lack of either the internal or external function of the pancreas. A still more important question is to what extent the hypertrophy was able to prevent or cure diabetes. In some instances the new formed tissue appeared functionally equivalent to the old, for not only was diabetes checked, but also to reproduce it a further resection of tissue was necessary to reduce the remnant to the size requisite in original operations. On the other hand, diabetes sometimes stopped when there was little or no hypertrophy of the remnant; also hypertrophy was frequent with continuance of diabetes. Closer study of these results, which appear contradictory at first glance, brings out the following explanatory facts. (*a*) The traumatic inflammation following operation is generally a factor in producing diabetes in cases with fairly large remnants. When by any means symptoms are prevented until the inflammation has subsided, it is sometimes found that the animal is no longer diabetic even though there has been little or no increase in the size of the remnant. (*b*) Inflammation may injure chiefly the islands, to such an extent that the animal remains diabetic after the inflammation has subsided notwithstanding hypertrophy of the remnant. If a functional injury may be assumed, as seems to be true in human diabetes, an explanation is afforded of the existence of diabetes even when the hypertrophic remnant contains numerous islands, as in Dog 151,[6] but this point is still doubtful. (*c*) Overfeeding furnishes the most

frequent explanation, and may be put to valuable use. For example, severe acidosis is obtainable only when the dogs can digest large quantities of fat, and dogs with small pancreas remnants can seldom do this. But by cautious overfeeding with carbohydrate after operation, mild diabetes may be kept up, while in a certain proportion of animals marked hypertrophy of the remnant is taking place. Islands are thus injured or destroyed, while the great increase of acinar tissue enables a satisfactory digestion of fat.

Age of Animals.—The animals mentioned above were fairly young, mostly 2 or 3 years of age. But both hypertrophy and its absence have been noted in dogs at all ages. Dog 146[7] was senile, yet the pancreas remnant in 3 weeks increased from 2.8 gm. to 6.3 gm. Also the records of puppies in a subsequent paper indicate no remarkable regenerative activity, just as the damaged pancreas of diabetic children fails to recover to any greater extent than that of adults.

Time Element.—It is impossible to determine by weight the beginning of hypertrophy, because the operation ordinarily is followed by inflammatory exudate and infiltration. Epithelial hyperplasia begins almost simultaneously and sometimes proceeds with astonishing rapidity. The most rapid increase observed was in Dog 152; namely, the growth of an estimated 4.4 gm. remnant to 10.4 gm. in 11 days. The hypertrophy seems to be mostly complete within the first few weeks after operation. The result is permanent, as shown in observations covering 3 years and over.

Cause of Hypertrophy.—The occasional hypertrophy of a mass of pancreatic tissue to several times its size, in contrast to the relatively slight increase in the great majority of instances, seemed a phenomenon worthy of attention for possible application in the treatment of human diabetes. Various attempts, by trimming remnants in different shapes, by multiple incisions through the surface, and by leaving protruding ducts as possible origins of proliferation, gave no consistent results. Here, as in certain of the preceding paragraphs, it is necessary to anticipate some of the findings of the microscopic study. The recently traumatized tissue shows a mass of inflammatory, degenerative, and regenerative changes, and it is inferred that the predominance of any of the three determines the result.

There is no warrant for attempting to stimulate regeneration of the human pancreas by mere trauma. Some more hopeful method may ultimately be worked out. The observations are important as confirmation of other evidence of the regenerative power of the pancreas in postembryonic life.

Repeated Operations.

In Table III, it was possible only to pick out the maximum weight of the pancreas remnant with which different animals developed diabetes in single operations. Each animal in Table IV was subjected to successive removals of pancreatic tissue, so that the exact reduction of the organ requisite for diabetes was sometimes determined within a fraction of a gram. The results in general confirm those of Table III. The traumatic inflammation of repeated operations accounts for the occasional occurrence of diabetes with large remnants. The degree of hypertrophy, obtained as a total by adding together the weights of tissue removed in operations and present at autopsy, was similar to that observed with single operations.

The observations were mostly long, generally continuing for months or years after the last operation. The operations were from two to five in number, and the intervals between them ranged from 2 weeks to 32 months. The time elapsed made no demonstrable difference in the susceptibility to diabetes. This conclusion has reference to the suggestion of some former writers that animals may develop "immunity" to the lack of pancreatic tissue, particularly by vicarious action of other glands. Such ideas are probably explained by the fact that these workers dealt with atrophic pancreas remnants lacking duct communication with the bowel, so that (*a*) in subsequent operations some fragments were missed, and (*b*) cachexia hindered the development of typical diabetes.

The same observation supports the conclusion of papers to follow that partially depancreatized animals show no inherent increase of tendency to diabetes with time.

TABLE IV.

Repeated Operations.

Dog No.	Normal body weight.	Total pancreas weight.	Remnant.		Hypertrophy.	Time elapsed between first and last operations.	Remarks.
			Estimated weight.	Fraction.			
	kg.	*gm.*	*gm.*		*gm.*	*mos.*	
B2–02	10.5	30.5	6.8	$\frac{1}{4}-\frac{1}{5}$		32	Very mild diabetes, due to traumatic inflammation.
B2–05	6.5	11.5	1.2	$\frac{1}{8}-\frac{1}{9}$		$\frac{1}{2}$	Mild diabetes, stopped by distemper.
B2–43	11.1	18.8	5.1	$\frac{1}{4}$	5.1–5.8	$\frac{4}{6}$	Slow onset of mild diabetes.
B2–51	9.75	26.2	2.75	$\frac{1}{9}-\frac{1}{10}$		1	Severe diabetes.
B2–53	10.25	24.5	3.5	$\frac{1}{7}$	3.5–4.5	$\frac{4}{6}$	Cachexia.
B2–63	24.4	49.0	7.4	$\frac{1}{6}-\frac{1}{7}$	7.4–14.2	22	Diabetes resulted from subsequent operations removing 1.32, 0.36, and 0.22 gm.
B2–88	14.4	30.7	2.8	$\frac{1}{11}$	2.75–2.75	$1\frac{1}{2}$	Subcutaneous graft also present. Diabetes followed its removal.
B2–89	13.1	30.1	2.7	$\frac{1}{11}$		11	Diabetes checked by emaciation; restored by removal of additional 0.65 gm.
C3–20	24.0	51.9	11.5	$\frac{1}{4}-\frac{1}{5}$	11.5–20.2	$4\frac{1}{3}$	Mild diabetes after four subsequent operations removing total of 5.3 gm. Remnant at autopsy weighed 14.9 gm.
C3–27	16.25	38.4	4.4	$\frac{1}{9}$	4.4–8.7	8	Severe diabetes after removal of additional 0.9 gm.
C3–45	10.8	27.5	3.7	$\frac{1}{7}-\frac{1}{8}$		$4\frac{1}{2}$	Transitory diabetes; permanent after removal of additional 0.3 gm.
C3–58	15.9	43.8	2.9	$\frac{1}{15}$	2.9–4.25	$4\frac{1}{3}$	Diabetes controlled by diet. Dog emaciated to 9 kilos.
C3–86	15.0	35.9	2.9	$\frac{1}{12}-\frac{1}{13}$	2.9–1.8	$7\frac{1}{12}$	Anorexia prevented diabetes till additional 0.2 gm. was removed. Sclerosis and atrophy of remnant.
C3–98	13.4	27.9	2.1	$\frac{1}{13}$	2.1–4.4	8	Diabetes controlled by diet. Later two operations removing total of 1.45 gm. necessary to bring back diabetes.
D4–63	15.25–18.7	46.5	4.6*	$\frac{1}{10}$		$7\frac{2}{6}$	Mild diabetes.
D4–85	10.0	25.9	2.0	$\frac{1}{13}$		1	Severe "
D4–86	15.9	35.5	3.9	$\frac{1}{9}$	3.9–10.8	$1\frac{1}{2}$	" "
D4–90	36.9	82.8	1.9	$\frac{1}{42}-\frac{1}{43}$		1	" "
D4–96	11.6	24.2	1.8	$\frac{1}{13}-\frac{1}{14}$	1.8–2.4	$3\frac{2}{3}$	" "
E5–00	15.25	35.0	3.5	$\frac{1}{10}$		$\frac{5}{6}$	" "
E5–16	18.0	34.0	6.75	$\frac{1}{5}$	6.75–9.0	$\frac{2}{3}$	Mild "

*Weight at autopsy.

Pancreas Weight in Relation to Reduced Body Weight.

The filling of the acini with zymogen during short fasts and the discharge on functional stimulation are probably sufficient to cause considerable changes in the gross weight. On the other hand, long fasting is accompanied by shrinking of the acinar cells and diminution

TABLE V.

Dogs with Malnutrition.

Dog No.	Normal body weight.	Final body weight.	Pancreas weight.	Weight of pancreas per kilo of normal body weight.	Weight of pancreas per kilo of final body weight.	Remarks.
	kg.	*kg.*	*gm.*	*gm.*	*gm.*	
C3–17	12.6	10.5	22.2	1.76	2.11	Poor appetite for 3 wks. 110 gm. of glucose subcutaneously 3 hrs. before death.
D4–27	12.5	8.8	22.1	1.77	2.51	Overfatty diet for 3 mos. Fat intoxication. 40 gm. of glucose subcutaneously 6 hrs. before death.
D4–31	10.6	8.0	12.1	1.14	1.51	Fasting for 2 wks., followed by overfatty diet for 6 wks. Fat intoxication.
F6–54		5.6	24.9		4.45	Thin when received. Killed with phosphoric acid intravenously.
F6–95	4.3	3.7	8.5	1.98	2.30	Fasting for 12 days; poor appetite following 12 days. Death from acetoacetic acid intravenously.
G7–21		15.0	30.6		2.04	Very thin when received.
G7–87		6.4	14.7		2.30	" " " " Killed with bicarbonate intravenously.
G7–89	18.0	14.0	47.8	2.66	3.41	Fasting and phlorizin 1 wk.; appetite poor 1 wk.; then killed with acid and alkali intravenously.
G7–99		8.0	15.0		1.87	Thin when received. Death from shock and bicarbonate.
C3–01		14.9	29.6		1.99	Collie; very thin when received.

of zymogen, so that in some cases the pancreas consists entirely of involuted rounded cells, with only rare traces of zymogen and little or no visible acinar arrangement.[8] The gross organ is softer and smoother, less lobulated, sometimes almost translucent or gelatinous

[8] Allen,[2] Chapter XXI, also Figs. 2, 3, and 4.

in areas, and obviously below normal weight. The present series did not include extremely short or long fasts, but only such intermediate degrees of abstinence or reduced diet as caused appreciable reduction of body weight.

Table V is made up of dogs which were either received in a very emaciated condition or suffered more or less loss of weight under

TABLE VI.

Fasting Dogs.

Dog No.	Normal body weight.	Final body weight.	Pancreas weight.	Weight of pancreas per kilo of normal body weight.	Weight of pancreas per kilo of final body weight.	Remarks.
	kg.	*kg.*	*gm.*	*gm.*	*gm.*	
C3–15	14.5		17.3	1.19		Long voluntary fast. 150 gm. of glucose by stomach 1¼ hrs. before death
D4–01	14.5	10.6	22.8	1.57	2.15	Undernutrition for 54 days, followed by fasting for 4 days.
F6–50	6.0	4.2	16.7	2.78	3.98	Fasting for 11 days with occasional doses of hydrochloric acid.
F6–52	6.3	5.0	13.4	2.13	2.68	Fasting for 8 days. Killed with acid sodium phosphate intravenously.
F6–79	10.2		23.1	2.26		Fasting for 20 days with large doses of hydrochloric acid.
F6–99	6.4	4.5	12.2	1.91	2.71	Fasting for 17 days. Death from 20 gm. of lactose intravenously.
G7–00	5.5	3.5	13.6	2.47	3.89	Fasting for 25 days. 25 gm. of glucose intravenously on last day. Milk vomited.
G7–01	5.5	4.4	10.3	1.87	2.34	Fasting for 18 days. Death from dextrin intravenously.
B2–52	12.8	9.9	23.5	1.84	2.38	Fasting for 16 days before operation.
B2–57	11.1	8.6	21.6	1.95	2.51	" " 18 " " "
B2–58	11.8	9.1	18.0	1.53	1.98	" " 18 " " "

observation. The animals of Table VI underwent actual fasting for various periods, sometimes together with other experimental procedures as noted. Those of Table VII received phlorizin as stated, subcutaneously, besides the fasting or undernutrition imposed.

In a majority of instances, both the absolute and relative weights of the pancreas fall within the normal extremes shown in Table II,

TABLE VII.

Fasting or Undernutrition with Phlorizin.

Dog No.	Normal body weight.	Final body weight.	Pancreas weight.	Weight of pancreas per kilo of normal body weight.	Weight of pancreas per kilo of final body weight.	Remarks.
	kg.	*kg.*	*gm.*	*gm.*	*gm.*	
C3–54	19.5	15.0	20.5	1.05	1.37	Fasting with 1 gm. of phlorizin daily for 10 days. Lard feeding and bicarbonate intravenously on day of death.
C3–57	14.5		13.9	0.96		Fasting for 9 days; pure fat feeding on last 3 days. 1 gm. of phlorizin daily.
C3–69	17.5	13.9	23.0	1.31	1.65	Undernutrition, followed by fasting for 5 days with 1 gm. of phlorizin daily. Glucose intravenously on day of death.
C3–99	22.5	17.0	31.2	1.39	1.83	Protein-fat diet with 0.5 or 1 gm. of phlorizin daily for 50 days.
D4–00	13.2	10.8	17.9	1.36	1.66	Protein-fat diet with 0.5–0.75 gm. of phlorizin every day or two for 50 days. Glucose and bicarbonate on day of death.
E5–07	15.0	11.4	30.8	2.05	2.70	Fasting for 10 days with four doses of 1 gm. of phlorizin. 50 gm. of glucose by stomach 2 days before death.
E5–33	17.5	13.9	35.0	2.00	2.52	Fasting for 1 wk. with five doses of 0.5 gm. of phlorizin.
F6–22	13.6	9.8	31.7	2.33	3.24	Fasting for 10 days with 0.5 gm. of phlorizin daily. 50 gm. of glucose subcutaneously on day before death.
F6–25	12.0	7.1	18.5	1.54	2.63	Fasting for 11 days with three doses of 0.5 gm. of phlorizin.
F6–32	17.6		27.0	1.53		Fasting for 6 days with three doses of 1 gm. of phlorizin. Bicarbonate on last 2 days.
F6–33	14.2		22.7	1.60		Fasting for 9 days. Bicarbonate on 2 days before death, and sodium chloride on 2 days before that.
F6–67	16.0	11.0	31.1	1.94	2.83	Fasting for 16 days with seven doses of 1 gm. of phlorizin.
F6–91	12.7	9.0	20.3	1.60	2.26	Partial nephrectomy. Fasting for 15 days with four doses of 1 gm. of phlorizin. Fed meat on 11th day of fast. 25 gm. of glucose subcutaneously on 13th day.

TABLE VII—*Concluded.*

Dog No.	Normal body weight.	Final body weight.	Pancreas weight.	Weight of pancreas per kilo of normal body weight.	Weight of pancreas per kilo of final body weight.	Remarks.
	kg.	*kg.*	*gm.*	*gm.*	*gm.*	
G7–28	4.5		10.9	2.42		Fasting for 5 days with three doses of 0.5 gm. of phlorizin. Butyric and acetoacetic acids intravenously.
G7–75	12.6	11.5	18.5	1.47	1.61	Fasting for 5 days with two doses of 1 gm. of phlorizin.
G7–76	11.2	9.0	20.4	1.82	2.26	Fasting for 8 days with four doses of 1 gm. of phlorizin. Early pregnancy and abortion.
G7–82	15.0		24.8	1.65		Fasting for 7 days with three doses of 1 gm. of phlorizin.
G7–93		11.4	23.7		2.08	Fasting for 4 days with one dose of 0.5 gm. of phlorizin on 1st day.
G7–96	11.0	10.0	25.5	2.32	2.55	Fasting for 5 days with two doses of 0.5 gm. of phlorizin.

whether calculated on the normal or the reduced body weight. Also in a majority of instances, they are closer to the average for the reduced than for the normal body weight. The rule has such marked exceptions in both directions that a table of averages could not properly be used. The degree of undernutrition is as great as any employed for controlling even the severest diabetes, and the results do not warrant a conclusion that the therapeutic effects of undernutrition are explainable by an increase of the ratio of pancreas mass to body mass. The islands perhaps shrink less than the acinar tissue in fasting, but there is no basis for a conclusion concerning mass relations.

Any marked changes in the mass of the pancreas due to accompanying experiments would also be revealed by such comparisons. For example, the exceptionally large pancreas weights of Dogs F6–54 and G7–89 in Table V represent the edema of the pancreas sometimes produced by acid injections. The other experimental procedures, such as glucose injections and especially phlorizin poisoning, did not demonstrably alter the gross weight of the pancreas.

EXPERIMENTAL STUDIES ON DIABETES.

Series I. Production and Control of Diabetes in the Dog.

2. Effects of Carbohydrate Diets.

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The belief underlying the dietetic treatment of diabetes has been that the disorder consists in the weakness of a bodily function, which is broken down by overstrain and spared or strengthened by rest. A coexistent and equally firmly rooted belief has been that some or perhaps most human cases are inherently progressive, on account of continuing toxic, infectious, or undetermined factors, so that they become worse and lead to death after a longer or shorter time in spite of dietary restrictions. Attention was previously directed[1] to the importance of determining this point positively in dogs, which are free from constitutional tendencies. This fundamental question of the possibilities and limitations of injuring assimilation and shortening life by overfeeding and improving tolerance and lengthening life by restricted diet was not fully settled by the experiments of Thiroloix and Jacob[2] or the present writer, and papers to follow will be devoted particularly to this subject. Microscopic changes will be mentioned briefly here and treated more completely later.

Effect of Starch-Rich Diet.

Dog B2–65.—Female; mongrel; black, slightly shaggy; age 5 years; rather thin; weight 17.5 kilos. May 25, 1914. Removal of pancreatic tissue weighing 28.9 gm.[3] Remnant about main duct estimated at 3.4 gm. ($\frac{1}{9}-\frac{1}{10}$). The urine

[1] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913, Chapter X.

[2] Thiroloix, J., and Jacob, *Bull. et mém. Soc. méd. hôp. Paris*, 1910, xxix, series 3, 492; xxx, series 3, 29, 656.

[3] All operations were performed under ether anesthesia.

remained sugar-free on fasting till May 29. Bread and soup diet was then begun, and glycosuria continued absent till June 2, after which date it was continuous. Bits of pancreatic tissue were removed by operations on June 15 and July 9 without apparently altering the steady downward progress. Glycosuria increased; at the outset it was as low as 1.5 per cent in 200 cc. of urine for 24 hours, at the end as high as 9.1 per cent in 2,415 cc. of urine. From July 23 to 28, the sugar was only once below 14 per cent, being thus exceptionally high in percentage. The animal remained in good spirits, but the weight diminished to 9 kilos, with corresponding loss of strength. Death occurred from weakness on July 29.

Autopsy.—Except for the emaciation, the autopsy was entirely negative. The pancreas remnant weighed 3.35 gm. The samples of pancreatic tissue for June 15, July 9, and autopsy showed normal acini and successive stages of hydropic degeneration of islands, involving finally a marked reduction in number and size of islands but by no means complete disappearance.

This record may be considered typical of the rapidly fatal course of this form of experimental diabetes on starch-rich diet, corresponding to the rapid emaciation and death in the severest human cases when entirely untreated, though the process is generally more rapid in dogs than in patients.

Breaking Down Tolerance with Glucose.

Dog B2–31.—Female; bull-terrier; white; age 2 years; good condition; weight 11 kilos. Dec. 23, 1913. Removal of pancreatic tissue weighing 19 gm. Remnant about main duct estimated at 2.6 gm. ($\frac{1}{8}$–$\frac{1}{9}$). Subsequently, bread feeding caused only a fraction of a per cent of glycosuria, which ceased and remained absent even on addition of glucose, until the quantity of glucose reached 200 gm. daily. Glycosuria ranging from 2 to 5.5 per cent in the mixed 24 hour urine of each day continued on the diet of bread and soup with glucose as stated till Feb. 12, 1914. The diet was then changed to meat *ad libitum*. Glycosuria steadily diminished during the succeeding days, till on Feb. 20 it was only 0.22 per cent and was evidently on the point of disappearing. The bread and glucose diet was repeated, with heavier glycosuria than before (up to 5.8 per cent in 24 hour urine of 2,470 cc.), until Feb. 26. The diet of meat *ad libitum* was then resumed, with the result that glycosuria fell as low as 0.9 per cent in 367 cc. of urine on Feb. 28, but rose to 6 per cent in 1,090 cc. of urine on Mar. 6. The condition thereafter was severe diabetes, and the animal was used for therapeutic tests, stringent regulation of diet and weight being necessary to keep glycosuria absent.

This animal was specially suited for the purpose because of an unusually voracious and unfailing appetite and a natural fondness for sweets. Most dogs will not eat so much bread and sugar for so long

a time. Animals and patients alike can evidently bring on diabetes by gluttony, particularly in starch and sugar, when a certain degree of predisposition exists.

Indigestion and diarrhea check the carbohydrate excess and prevent diabetes in many animals, and strong digestive power facilitates the production of experimental and doubtless also of clinical diabetes. Talcum powder mixed with the food, in quantities up to 100 gm. or more daily, has been the chief means used to control diarrhea. The innocuousness of talcum in any amount or duration for such experiments suggested its introduction later as a flour for making pancakes for some patients on extremely low diets.

The downward progress of human diabetes can be further imitated in that the disease in dogs can be made slow or rapid as desired by varying the kind and quantity of food. Like human patients these dogs never regain a tolerance for food equal to that which they possessed before the damage from overfeeding occurred.

The plan of breaking down tolerance with the largest feasible quantities of starch and glucose immediately after operation is of practical importance for securing diabetic dogs with the largest possible pancreas remnants, and hence with the best possible digestive power and vigor. Knowledge of animals and skill in managing their diets are important for success in producing the most valuable test objects for diabetic research; namely, dogs which eat and digest well and appear normal in all respects except for the desired degree of diabetes. The best results require that the investigator should give personal attention to this work.

Dog B2–80.—Female; mongrel; black and white; age 3 years; very good condition; weight 17 kilos. Nov. 17, 1914. Removal of pancreatic tissue weighing 25.7 gm. Remnant communicating with both ducts estimated at 7.1 gm. ($\frac{1}{4}$–$\frac{1}{5}$). The dog was specially valuable, because with this large pancreas remnant immediately after operation she had a tendency to slight glycosuria on plain bread and soup diet, and was easily kept glycosuric by the addition of small quantities of glucose. After a period of sugar freedom on meat diet, the dog was loaned to another laboratory, and was returned with the statement that she could not be made diabetic. Trial with increasing dosage of glucose showed that glycosuria actually remained absent with such quantities as could voluntarily be eaten, and there was danger here as in many cases with large remnants that spontaneous recovery of assimilation due to the delay might spoil the opportunity for diabetes.

Accordingly, beginning Apr. 24, 1915, 500 gm. of glucose were given daily. Diarrhea was controlled by the use of bone-meal and talcum powder, and any portions of the sweetened bread and soup mixture left uneaten were made into firm balls and fed forcibly. Only a 5 day period of this excessive dosage was necessary before it became possible to reduce the quantities with continuance of glycosuria. The animal was later overfed with protein and fat so as to bring on severe diabetes and acidosis. Aug. 14. Died in coma.

Autopsy.—The dog possessed abundant fat at death, like some human patients dying of acidosis. The body weight was 17.25 kilos, the weight of the pancreas remnant 13.5 gm. Its tissue was soft, normally lobulated and free from fibrosis, but microscopically consisted of unbroken expanses of acini. Rare small clumps, apparently of alpha cells, were the only remains of islands. Many small ducts showed appearances like vacuolation.

Unsuccessful Attempts to Break Down Tolerance.

Dog B2–02.—Female; Boston terrier; brindle; age 1½ years; weight 10.5 kilos. This dog was received Oct. 25, 1913, and was used for various experiments, especially the removal of successive portions of pancreas tissue, until an operation on Dec. 10, 1914 at length produced diabetes. Glycosuria was present up to Dec. 23 on plain bread and soup feeding. It could readily be produced thereafter by the addition of glucose. But the dog was exceptionally dainty in appetite, would eat only limited quantities of bread, had a strong distaste for sugar, and vomited readily and persistently if forcibly fed. The potential diabetes continued, as proved by the easy production of glycosuria by occasional glucose feedings and the low tolerance for glucose subcutaneously, but active symptoms could never be maintained. Aug. 31, 1915. A bit of pancreas tissue was removed for examination. The condition thereafter was unchanged, namely absence of glycosuria with bread diet, also with the addition of 100 gm. of glucose, but glycosuria with the addition of 200 gm. of glucose; and all such attempts were terminated within a few days by refusal and vomiting. On mixed diet of bread and meat the dog continued in full health at the original weight of 10.5 kilos, until death from rabies, Sept. 19, 1916.

Of numerous animals in which the determining influence of appetite upon the occurrence of diabetes was observed, this one afforded the most striking antithesis to Dog B2–31 above mentioned. The actual assimilative power was much lower, in consequence of operation, in this dog than in Dog B2–31, and the eating habits were alone responsible for the opposite results.

Autopsy.—The pancreas remnant weighed 6.8 gm. Grossly it was normal in appearance and consistency, and microscopically the acini were fully normal; but occasional small fibrous trabeculæ and a general scarcity of islands indicated a previous inflammation as the probable cause of the potential diabetes with such a large remnant of pancreatic tissue. Also the island cells, both on Aug. 31, 1915, and at autopsy, showed numerous suggestions of vacuolation, generally so slight

as to be doubtful. These anatomic findings are of importance in connection with the question discussed below of the probable ultimate outcome of such a diet in such an animal.

Dog B2–86.—Male; short haired mongrel; cinnamon and black; very old, obese, and phlegmatic, but vigorous and active; weight 32.4 kilos. Apr. 7, 1914. Removal of pancreatic tissue weighing 48.9 gm. Remnant about main duct estimated at 12.4 gm. ($\frac{1}{4}$). The dog was inordinately greedy, but had no glycosuria even from the largest amounts of bread and soup which he ate. Apr. 19. This diet was increased by 250 gm. of glucose, with resultant glycosuria of 1.25 per cent, which steadily diminished to 0.2 per cent on Apr. 23. The glucose was then increased to 500 gm. daily, but nevertheless glycosuria was absent thereafter. It was possible to continue heavy glucose feeding with only a few days of intermission to avoid anorexia, indigestion, or diarrhea. The bread was ordinarily not weighed, but on certain test days the dog eagerly ate as much as 1 kilo of dry bread and 800 gm. of glucose. The average bread ration was nearer 500 gm., but 400 to 600 gm. of weighed glucose were taken with this. No other dog in the series was willing to eat or able to digest such quantities of carbohydrate, and advantage was taken of this peculiarity in order to test whether diabetes might ultimately result from such a load upon the carbohydrate metabolism. Glycosuria was rare and slight. Gain in tolerance was also indicated by tests on May 4 and 13. On each of these dates the dog was fed 400 gm. of bread, 200 gm. of glucose, and 500 cc. of soup, and the urine and plasma sugars were followed as shown in the accompanying table.

Dog B2–86.

Date.	Time.	Plasma sugar.	Urine.		Date.	Plasma sugar.	Urine.	
			Volume.	Sugar.			Volume.	Sugar.
1914		*per cent*	*cc.*	*per cent*	*1914*	*per cent*	*cc.*	*per cent*
May 4	Before feeding.	0.089	376	0	May13	0.099	123	0
	½ hr. after feeding.	0.196	12	Faint.		0.196	6	0
	1½ hrs. " "	0.286	15	0.52		0.156	5	0
	2½ " " "	0.298	21	0.52		0.192	2 drops	Faint.
	3½ " " "	0.357	14	1.76		0.232	25	0.62
	4½ " " "	0.476	80	3.53				
	6 " " "	0.435	75	3.61		0.285	25	1.22
	8 " " "	0.400	95	3.22		0.270	25	1.13
	9 " " "	0.270	55	2.22		0.286	35	1.00
	11 " " "	0.294	45	1.81		0.250	75	0.55
	13 " " "	0.263	3	0.96				
	14 " " "	0.156	100	0.70		0.176	110	Faint.
	16 " " "	0.099	40	0		0.098	155	0
	24 " " "	0.062	143	0		0.098	320	0

The poor prospect of overcoming the increased tolerance by higher doses of carbohydrate was indicated by a shorter test on July 21.

Dog B2–86.

Date.	Time.	Plasma sugar.	Urine sugar.	Remarks.
1914		*per cent*	*per cent*	
July 21	Before feeding.	0.124	0	Fed 400 gm. of bread with 500 gm. of glucose.
	2 hrs. after feeding.	0.147	0	
	7 " " "	0.141	0	

Aug. 7. Removal of a bit of pancreas was attempted. The remnant was excessively congested; stubborn oozing prolonged the operation a little, and the dog died under the operation. The pancreatic congestion was not necessarily associated with the sugar feeding, for it has been found in occasional dogs under other conditions. The easy death from ether or shock may have been due to injury from the glucose, though the animal seemed as well and strong as ever, or merely to senility and obesity.

Autopsy.—The pancreas remnant weighed 24.8 gm. and the tissue including the islands was normal in all respects. The hypertrophy of the remnant was thus accompanied by recovery from diabetes to such an extent that no apparent injury resulted from the most excessive gluttony in carbohydrate for 4 months. The intact state of the islands was evidence that more prolonged carbohydrate excess could not have caused a lowering of tolerance, which is due to hydropic degeneration of the islands as formerly described.

Repeated Operations.

It is a familiar and readily comprehensible experience that glycosuria may be absent or transitory on the highest possible starch and sugar feeding, and that the diabetes which could not be induced by diet is brought on by the removal of an additional tiny fragment of pancreas. An example was previously given.[4] Others are mentioned in the preceding paper.[5] It is there seen that 0.3 gm. of tissue in the case of Dog C3–45, or 0.22 gm. in the final operation of Dog B2–63, sufficed to make the difference between diabetes and its absence. Certain points will be further developed by giving a summary of individual protocols.

[4] Allen,[1] p. 486, Dog 176.

[5] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 375, "Remarks" column of Table IV.

Dog B2–63.—Male; mongrel; brown and white, shaggy; age 6 years; slightly thin; weight 24.4 kilos. May 22, 1914. Removal of pancreatic tissue weighing 41.6 gm. Remnant about main duct estimated at 7.4 gm. ($\frac{1}{6}$–$\frac{1}{7}$). Glycosuria was continuously absent on a diet of beef lung up to June 23. The diet was then changed abruptly to bread and soup. Glycosuria of 0.2 per cent in 1,140 cc. of urine was present on June 24, but ceased immediately. June 29. 100 gm. of glucose were added, with resultant glycosuria of 0.52 per cent in 1,185 cc. of urine; this also ceased immediately. Similar transitory traces of glycosuria followed the increase to 200 gm. of glucose on July 1, to 300 gm. on July 8, and to 400 gm. on July 30. On Aug. 3 (after more than a month of continuous bread and glucose diet) the attempt to produce diabetes by feeding was abandoned, and the dog was used for phlorizin experiments.

Nov. 6. An additional 1.32 gm. of pancreatic tissue was removed, the body weight being then 23.9 kilos. Nov. 23. The urine was still sugar-free on bread and soup diet; 100 gm. of glucose were given by stomach tube before feeding. This procedure caused moderate glycosuria on this day, less on the next day, and none on the following days. Nov. 28. The glucose feeding was stopped. Thereafter on plain bread and soup diet, slight to moderate sugar reactions appeared on Dec. 1, 8, 9, 10, 12, and thenceforth continuously to Jan. 3, 1915. The occurrence of glycosuria at first was governed by the quantities of bread eaten, the dog being hungrier on certain days. This lowering of tolerance on prolonged carbohydrate diet took place notwithstanding a loss of body weight to 22.7 kilos on Dec. 12. Glycosuria ceased with a single fast day on Jan. 3, and thereafter was continuously absent on a diet of beef lung *ad libitum.* Mar. 30. Weight 22 kilos; the only food given was 200 gm. of bread (weighed dry, and moistened with soup before feeding). The result was glycosuria of 0.87 per cent in 860 cc. of urine. Several other tests as mentioned below, to compare the assimilation of different starches, indicated a similar lowering of tolerance. As the dog did not thrive on the monotonous lung diet, the weight gradually fell, reaching its lowest level of 17.9 kilos on June 26. Corresponding to this marked decline in weight was a rise in tolerance, so that 200 gm. of bread no longer caused glycosuria; also on July 6, at a body weight of 18.75 kilos, the sugar in the blood plasma after ingestion of this quantity of bread rose only to 0.143 per cent. The diet was then changed to lung and suet *ad libitum;* the body weight gradually rose to 27 kilos in Aug. and Sept. The dog proved able to take 200 gm. of bread in addition to the usual diet, and on Sept. 20 the diet was changed to bread and soup with suet. Glycosuria remained absent, with the exception of 0.34 per cent on Oct. 7 and 0.32 per cent on Oct. 10. The body weight gradually rose, until by the middle of Nov. the animal was obese at a weight of 30.9 kilos. Nov. 16. Slight glycosuria appeared, and increased on Nov. 17. With 1 day of fasting and 1 day of exercise, glycosuria ceased and remained absent, on a diet of bread and soup, while the body weight fell to 30 kilos.

Nov. 30. Weight 30 kilos; pancreatic tissue weighing 0.36 gm. was removed. During the ensuing week traces of glycosuria occurred on bread and soup diet, though the dog ate poorly. Fasting was then imposed from Dec. 7 to 16.

Dec. 16. Weight 24.25 kilos. The animal was given all the bread and soup he would eat. Heavy glycosuria appeared (hunger glycosuria), diminishing to faint glycosuria on the following day and remaining absent thereafter, except for a trace of 0.14 per cent on Dec. 31. The weight slowly fell to 23 kilos by the middle of Feb.

Beginning on Feb. 14, 1916, at this weight, 200 gm. of glucose were added to the bread and soup diet. On this day there was glycosuria of 0.37 per cent, which ceased immediately. Beginning Feb. 18, 100 gm. of lard were added daily to the bread and glucose diet. The weight rose to 24.7 kilos by Feb. 26, while glycosuria remained absent. On this date the dog was changed from the warm room to an outdoor cage. On the identical diet traces of glycosuria were continuous for a few days, as will be described in a subsequent paper. The weight was meanwhile falling to 23.4 kilos, and on Mar. 4 to 6 an increase to 400 gm. of glucose produced no more than 0.45 per cent glycosuria in 600 cc. of urine.

Mar. 7. Pancreatic tissue weighing 0.22 gm. was removed. Thereafter bread and glucose feeding maintained glycosuria without difficulty. Mar. 27. An additional 0.8 gm. of pancreatic tissue was removed. Apr. 2. Death occurred from peritonitis.

Autopsy.—The pancreas remnant weighed 12.3 gm.

Microscopically, the important feature was the existence of vacuolation of island cells throughout the series of pancreatic specimens, very slight and scarce in the operations of Nov. 6, 1914, Nov. 30, 1915, and Mar. 7, 1916, and decidedly more advanced on Mar. 27, 1916, and at autopsy.

The clinical course, especially with consideration of the shorter life of a dog, bears a resemblance to a prolonged mild case of human diabetes. The vicissitudes and fluctuations of tolerance might appear superficially as bizarre and accidental, but comparison with numerous other examples furnishes a rational explanation for each change.

The factors to be observed particularly as influencing the tolerance are the diet, the body weight, and the natural reparative power. In brief, it will be noticed that up to January, 1915, heavy carbohydrate feeding reduced the tolerance, even though the urine was generally sugar-free and even though the body weight fell. This lowering of assimilation was still perceptible on March 30, but with carbohydrate-free diet and further decline of weight, the tolerance in June and July was found higher, and in September the animal was able to live on bread without glycosuria. The body weight was then increased by

the addition of fat to the diet. Evidence of actual reparative power in the pancreas was afforded, for the improved assimilation continued even at higher weights than before. But when in November the animal became obese at a weight above 30 kilos, decided glycosuria appeared. The same combination is evident in the operation of November 30 and the subsequent fast. The loss of 0.36 gm. of pancreatic tissue was more than counterbalanced by the reduction to 24.25 kilos in weight; but the genuineness of the reparative process is proved by the higher tolerance and weight as compared with the preceding March.

The reparative process mentioned may be associated with the marked hypertrophy of the pancreas remnant. The recovery, however, was overbalanced by the subsequent removal of 0.36 and 0.22 gm. of additional pancreatic tissue. The newly formed tissue therefore could not be functionally equivalent to the original tissue.

Even though no obvious clinical injury seemed associated with the carbohydrate excess preceding November 6, 1914, or with the obesity of November, 1915, or with the carbohydrate excess prior to March 7, 1916, the microscopic examination demonstrated injuriously overtaxed island cells at all these times. The downward progress clinically would doubtless have been still more evident if it had not been checked at certain periods as mentioned, and also if the observations had been extended for a still longer time. This downward progress is fully comparable with that of mild human cases treated on the lax plan of merely keeping the urine sugar-free most of the time, and it cannot properly be called spontaneous.

It will bear repeating that experiments of this sort require prolonged careful attention to the diet and all other details. Care must be taken that the animal likes his food and eats it, that he does not suffer from diarrhea or other illness, and that the regularity of his program shall equal that of diabetic patients whom he is supposed to imitate. Even so, only a minority of dogs thrive under the conditions and are adaptable to changes of diet and weight as desired. Therefore they are generally obtainable only by trial and choice among a considerable series of dogs. The best ones, which prove capable of such a delicately balanced tolerance and such close reproduction of clinical conditions, represent much labor and are useful for many experiments.

Dog C3–27.—Female; mongrel; yellow; age 4 years; good condition; weight 16.25 kilos. July 8, 1915. Removal of pancreatic tissue weighing 34 gm. Remnant about main duct estimated at 4.4 gm. ($\frac{1}{5}$). The subsequent condition was mild diabetes. Glycosuria was repeatedly produced by feeding 50 or 100 gm. of bread, or on certain occasions by large diets of beef lung. Also at the time of these tests the weight was down to approximately 15 kilos. Beginning Oct. 1, the diet was 500 gm. of beef lung and suet *ad libitum*. Dec. 1 to 5. The addition of 100 gm. of bread caused daily glycosuria not above 0.42 per cent in 450 cc. of urine, the weight then being 19.8 kilos. On omitting the bread the glycosuria ceased. Beginning Feb. 14, 1916, the addition of 100 gm. of bread produced no glycosuria. Feb. 23. The diet was changed to bread and soup. Feb. 25. 100 gm. of glucose were added to this diet, causing glycosuria of 0.63 per cent in 940 cc. of urine for 1 day. Glycosuria then ceased and could not be restored, though the glucose dosage was 200 gm. on Feb. 27, 300 gm. daily from Feb. 28 to Mar. 4, and 400 gm. daily to Mar. 9, when the attempt had to be abandoned because of distaste for the bread and glucose mixture. The dog meanwhile had become obese, reaching the maximum weight of 21 kilos.

Mar. 9. An additional 0.9 gm. of pancreatic tissue was removed. Bread diet caused no glycosuria on the following days. Beginning Mar. 13, 200 gm. of glucose were added daily, with negative urine till Mar. 16, when heavy glycosuria suddenly appeared. This continued after glucose was discontinued on Mar. 21. The subsequent course was downward progress on mixed diet to death in coma on Apr. 22.

Autopsy.—The pancreas remnant consisted of normal appearing tissue weighing 7.8 gm. Islands of Langerhans were very large and abundant but maximally vacuolated.

In the tissue removed at operation on Mar. 9, the islands were free from any visible abnormality.

Genuine recuperation of function in the pancreas remnant was demonstrated by the double evidence of markedly increased carbohydrate tolerance at a higher body weight, up to March 9. In view of the facts that the dog's digestion could not bear further prolongation of the high glucose feeding, that the islands at that time showed no sign of vacuolation, and that the tendency to recovery was so manifest, it is improbable that diabetes could have been brought on by any kind or duration of feeding.

This recovery of function corresponded to a marked hypertrophy of the pancreas remnant, which doubled in size. The great abundance of islands found in numerous slides indicated that they underwent hyperplasia which fully paralleled that of the acini. Nevertheless, the functional recovery was completely negatived by the removal of

only 0.9 gm. of additional tissue. The new formed tissue was therefore not equal to the original tissue in functional capacity.

The reason for the recovery of assimilative power, to such an extent that a second operation was required, lay in the long interval of sugar freedom granted to the animal after the first operation. If the pancreas remnant is of the large size which is most desirable, a dog should be subjected to occasional periods of glycosuria to prevent the tolerance from rising too high. Only after the diabetic condition has been thus maintained for months or years can the animal be trusted to remain permanently diabetic without this precaution. The existence of a considerable recuperative power at the outset, and the feebleness or absence of this power in the later stages, correspond closely to the well known facts in human diabetes.

By comparison with numerous other dogs, it may safely be assumed that carbohydrate overfeeding after the first operation would have sent this animal rapidly into severe diabetes. A carbohydrate-free diet, with limited protein so as to keep the urine sugar-free, permitted recovery of tolerance, even though the fat ration was so high as to produce obesity. Provided that a recuperative power exists in the pancreas, such a diet evidently affords opportunity for recovery and is far less harmful than an excessive carbohydrate diet. This fact was abundantly demonstrated by the benefits of the classical treatment in human cases, and is confirmed by similar observations in dogs.

Dog C3–45.—Female; mongrel; yellow; age 4 years; moderately well nourished; weight 10.8 kilos. Nov. 19, 1915. Removal of pancreatic tissue weighing 23.8 gm. Remnant about main duct estimated at 3.7 gm. ($\frac{1}{7}$–$\frac{1}{8}$). Glycosuria remained absent with fasting up to Nov. 23. Feeding of bread and soup then brought on heavy glycosuria. Nov. 27. The diet was changed to beef lung; glycosuria progressively diminished, and was absent on Nov. 30. Dec. 1. Heavy sugar (4.45 per cent in 380 cc. of urine) returned on bread diet, then stopped after 2 days of lung diet. Dec. 6. The dog was loaned to another laboratory, where she remained for a time on diets which caused little or no glycosuria. Mar. 16, 1916. The dog was sent back with the report that she was not diabetic. On this date the feeding of bread and soup with 300 gm. of glucose caused excretion of 1.6 per cent sugar in 528 cc. of urine; but the appetite and digestion of the dog could not endure continuance of this sugar dosage, and glycosuria therefore remained absent till Mar. 27. The body weight during this time was 11.3 to 11.6 kilos.

Mar. 27. Pancreatic tissue weighing 0.3 gm. was removed. Glycosuria remained absent with fasting for 2 days. Mar. 29. Bread and soup diet caused faint glycosuria, which became heavy (4.4 per cent in 2,000 cc. of urine) on the addition of 50 gm. of glucose. After Apr. 2, it continued heavy on plain bread and soup diet, and the dog was kept in a condition bordering on glycosuria thereafter. Glycosuria was absent on restricted diet, but always returned promptly on feeding bread or too much meat, and there was no further tendency to any marked recovery of tolerance. The animal was sent to another laboratory on July 10.

The noteworthy feature is that the considerable recovery of tolerance in this case was overbalanced by the removal of 0.3 gm. of additional tissue, and that this tiny bit of pancreas made the difference between diabetes and its absence.

Dog C3–86.—Female; mongrel; brown; age 3 years; good condition; weight 15 kilos. Apr. 28, 1916. Removal of pancreatic tissue weighing 33 gm. Remnant about main duct estimated at 2.9 gm. ($\frac{1}{12}$–$\frac{1}{13}$). Appetite was poor and weight was lost rather rapidly after operation. Glycosuria was absent on bread and soup eaten in small quantities, but present on the addition of 50 gm. of glucose. Sugar freedom on bread diet continued till May 22. Thereafter, 100 gm. of glucose were given by stomach tube before feeding each day. There was glycosuria of 1.8 per cent in 670 cc. of urine on May 23, 0.42 per cent in 312 cc. of urine on May 24, and none thereafter. Failing appetite compelled the stopping of glucose on June 11. Perhaps because of larger eating of bread and soup, glycosuria reappeared on June 15, the body weight being 13.5 kilos.

On that date 0.2 gm. of pancreatic tissue was removed, and at operation it was estimated that no hypertrophy of the remnant had occurred. The specimen was normal microscopically except for vacuolation in a very few cells of the abundant islands. Subsequently the dog proved vigorous up to death on Dec. 12, 1917. The pancreas remnant was soft and appeared normal, and its low weight of 1.6 gm. was perhaps partly due to the fact that emaciation had reduced the body weight to 6.4 kilos.

The apparently high tolerance in this dog was due to poor eating and loss of weight. For the best diabetic experiments it is essential that the dogs should have good appetite and health in other respects.

Dog C3–98.—Male; mongrel; brindle; age 2 years; moderately well nourished; weight 13.4 kilos. June 8, 1916. Removal of pancreatic tissue weighing 25.8 gm. Remnant about main duct estimated at 2.1 gm. ($\frac{1}{13}$). Diabetes was controlled by diet, but its existence was demonstrated by the fact that during 2 months glycosuria could be produced at any time by feeding 100 gm. of bread.

The tolerance was spared by diet until Nov., when the body weight ranged slightly above and below 12 kilos. Bread and soup diet then failed to bring on glycosuria, and during the month Nov. 21–Dec. 21 the daily addition of 200 or 300 gm. of glucose also failed.

Dec. 21. Additional pancreatic tissue weighing 0.85 gm. was removed, the body weight being 11.25 kilos. The dog was unwell thereafter, with diarrhea and poor appetite, so that by Jan. 5, 1917, the body weight had fallen to 9.5 kilos. Glucose feeding as high as 300 gm. daily again failed to produce more than transitory glycosuria, though the dog began to regain weight. Feb. 7. Weight 11.4 kilos; the plasma sugar at 11 a.m. was 0.112 per cent. The usual bread and soup mixture with 300 gm. of glucose was then fed; glycosuria was absent, and at 4 p.m. the plasma sugar was 0.111 per cent.

Feb. 8. 0.5 gm. of additional pancreatic tissue was removed. Diabetes existed thereafter, so that glycosuria could be produced by plain bread and soup diet. June 4. Died; the dog was emaciated down to 8.6 kilos, and the pancreas remnant weighed 2.95 gm.

In the tissue removed Dec. 21, vacuolation of island cells was rare if present, and so slight as to be doubtful. Also on Feb. 8, no positive changes in the islands were demonstrated. The emaciation preceding death was due to other causes than diabetes, and there was no vacuolation of islands.

The long interval of freedom from glycosuria following the first operation permitted actual recovery from the diabetes. This was associated with hypertrophy of the pancreas remnant, and the new formed tissue seemed to be functionally equivalent to the old, for further resection was necessary practically to the same extent as in an original operation, even for an animal at the lower body weight. As usual, carbohydrate overfeeding did not cause degenerative changes in the islands of the non-diabetic animal, and the potential diabetes subsequently in absence of active symptoms was also attended with no island changes.

Glucose Intoxication.

Glucose overfeeding ordinarily causes no harm in dogs beyond diarrhea and loss of weight. In exceptional instances the attempt to produce diabetes has ended in death apparently from poisoning by the prolonged excess of sugar. Gastrointestinal disturbances have appeared sometimes as a prominent cause, and sometimes have been absent or trivial. Though these cases in such a resistant species as dogs are not positive, the genuineness of glucose intoxication is confirmed by fatalities in feebler animals, such as rabbits and monkeys, and in dogs weakened by a preceding Bernard puncture of the medulla.

Dog B2–48.—Female; mongrel; yellow and white; age 4 years; good condition; weight 14.75 kilos. Mar. 19, 1914. Removal of pancreatic tissue weighing 22.5 gm. Remnant about main duct estimated at 4.7 gm. ($\frac{1}{5}$). Glycosuria being absent on bread and soup diet, on Mar. 30, 200 gm. of glucose were added; the amount was increased on Apr. 1 to 300 gm. As the dog did not like this mixture, on Apr. 9 a mixture of chopped meat, bone-meal, and glucose was substituted, the latter in dosage of 200 or oftener 300 gm. daily. The dog held weight, without glycosuria, and seemed to be thriving until she was unexpectedly found dead on May 9.

Autopsy.—No cause of death was found and no organ changes beyond intense congestion of the liver. Though the animal had been dead several hours, 100 gm. of liver were taken in boiling potassium hydroxide, but the concentrated extract was negative for glycogen. Microscopically, the pancreas remnant (weight 6.8 gm.) and its islands were normal; the liver was strictly normal, except for intense congestion, and was not fatty; the other organs were negative. Death was due to some unknown intoxication.

Dog B2–69.—Male; bulldog; mongrel; white with black head; age 4 years; good condition; weight 19.9 kilos. May 29, 1914. Removal of pancreatic tissue weighing 36.3 gm. Remnant about main duct estimated at 9.8 gm. ($\frac{1}{4}$–$\frac{1}{5}$). The dog throve on bread and soup diet. July 8, 100 gm. of glucose were added, increased on July 15 to 200 gm., on July 20 to 300 gm., and on July 30 to 400 gm., the mixture being improved by the addition of a little chopped meat daily. The weight gradually diminished, but the dog remained lively. Oct. 20. Weight 14 kilos; there was weakness out of all proportion to the emaciation. The animal, though retaining appetite and spirits, could not stand. Glucose was discontinued and meat fed. Oct. 22. The dog was still unable to stand, though wagging his tail and making efforts to rise. On this day was noticed bloody diarrhea, which previously had either been absent or too slight to attract attention. Oct. 23. The animal was found dead.

Autopsy.—The pancreas remnant appeared normal and weighed 16.1 gm. The stomach and intestines appeared normal throughout except for a portion 3 feet in length beginning 6 feet from the pylorus, where the mucous membrane was deeply injected and ecchymotic. The liver and other viscera appeared normal without congestion. There was no noticeable excess or diminution of fluid in the brain or cord. Microscopically the liver, adrenals, and pancreas were found normal. As in Dog B2–48, no fat vacuoles were visible in routine stains of the liver.

Dog B2–49, subjected to similar prolonged glucose feeding, died of gas bacillus infection, which is rare in dogs. Experiments with infection of diabetic dogs with this organism will be published later, but were on the whole negative.

Comparisons of Carbohydrates.

Comparison of Starch and Glucose.—Numerous observations have made it evident that glucose brings on glycosuria and diabetes more actively than starch. The difference is not merely one of quantity, for the addition of 50 or 100 gm. of glucose is sometimes effective when no amount of bread feeding avails for glycosuria. The reason for the readier glycosuria from glucose lies evidently in its quicker absorption, and if distributed in sufficiently small doses throughout the day it would presumably be assimilated as well as starch, as Klemperer showed in human patients. The most important point here is that the sudden glucose flood with its attendant glycosuria is more injurious to the pancreatic function than the more gradual and prolonged labor imposed by starch. It may thus be inferred that sugar is a more dangerous food for human beings with any predisposition to diabetes than is starch.

The question may be raised whether the difference is not merely one of time, and whether starch will not bring on diabetes more slowly than glucose but just as surely. As the difference between the two is not very great, experiments need to be performed with full precautions that the body weight does not vary, that the preliminary tests of the glucose tolerance do not lower the tolerance for starch, and under other conditions of exactness. Examples have already been given in which bread feeding was tolerated for several days before onset of glycosuria. The feature which renders longer tests peculiarly difficult is the recuperative power of the pancreas, which through regeneration reduces the diabetic tendency sometimes to the vanishing point, as described in preceding protocols. This experimental difficulty strengthens the view of the greater danger of sugar for predisposed human individuals, because such power of regeneration as their pancreas may possess will evidently have a better opportunity on starch diet. The difficulty may by extreme care be overcome in very prolonged experiments, in which the animals are kept potentially diabetic for so long a time that the regenerative tendency largely disappears. These conditions were fairly well fulfilled in Dog B2–02, described above. This animal could have been made actively diabetic at any time by glucose except for her repugnance to it. The

feature of incompleteness in the experiment is that death occurred from rabies before diabetes actually ensued on starch diet, but the hydropic degeneration in the islands of Langerhans demonstrated sufficiently that loss of tolerance was in progress and diabetes inevitable.

The record of Dog B2–01 from Sept. to Dec., 1916, to be published later, also shows a downward progress on bread diet, though the diet at first was apparently assimilated even with the addition of 300 gm. of glucose. The same result is shown in the experiments with Dogs D4-52 and D4-69 in Paper 3 of this series.[6] In the former animal between Aug. 8 and 21, 1917, it was impossible to maintain glycosuria on bread diet with 200 gm. of glucose, and the sugar feeding had to be stopped because of the distaste acquired by the dog for it. The tests of blood and urine on Aug. 8 and 15 also indicated a rise of tolerance during this period. But with continuance of plain bread feeding, glycosuria appeared on Oct. 10, and in a test with the addition of 200 gm. of glucose to the usual bread mixture the excretion was as high as 7 per cent. Likewise Dog D4-69 displayed an apparently increasing assimilation, so that the animal finally tired of glucose and the attempt to maintain glycosuria apparently failed. But with continuance of bread feeding, glycosuria began on Oct. 6, and, just as with Dogs B2-01 and D4-52, the tolerance proved to be permanently lowered thereafter.

When the regenerative power of the pancreas is not so great as to result in a practical cure of the diabetes, the difference between starch and glucose seems to be essentially one of time and degree. Glucose through its quicker absorption floods the body more suddenly and violently, but starch works the same damage more slowly but just as surely. This was shown in several animals through the onset of diabetes, and in one by examination of the islands of Langerhans. By inference, if human diabetes is so mild that glycosuria ceases on withdrawal of sugar without limitation of other carbohydrate, it is not correct to treat the case in this manner or to leave either starch or total calories unrestricted.

When the regenerative power of the pancreas is sufficient, there is a real difference due to the time element mentioned, and a number of examples have already been given of more or less complete recovery from diabetes on starch diet by dogs which could readily have been made diabetic by forcing of glucose shortly after operation. Some-

[6] Allen, F. M., *J. Exp. Med.*, 1920, xxxi (in press).

thing similar may conceivably occur in some human patients. In properly chosen animals without too strong a tendency either to diabetes or to recovery, it is possible to demonstrate a recuperative attempt by functional tests showing an apparent increase of assimilation, but the continued overtaxing of the assimilation causes its breakdown, and recovery to the former extent is never again possible. A similar gain of tolerance is well known in human patients under dietary restriction in the earlier stages of diabetes, and the warning against abusing this recuperative tendency is similar, especially as actual recovery on the part of human patients has seldom been witnessed.

Three of the four dogs here mentioned were kept sufficiently long to demonstrate that the lowering of tolerance referred to was actually the result of the carbohydrate excess and not of any inherent tendency in the dogs. The onset of diabetes was evidently checked by change to protein-fat diets, and especially in Dog B2–01 the tolerance could be manipulated up and down by changing the body weight. It is therefore confirmed that carbohydrate injures the assimilation more rapidly and powerfully than any other food. The later observations show also the harmfulness of luxus diets, but these diets are most dangerous when they include carbohydrate.

Comparisons of Starches.

Experiments were begun to test the glycosuric effect of different starchy foods, in relation to the hypotheses of certain writers concerning carbohydrate "cures." Dogs with potential diabetes of long standing and with fairly stationary assimilative power were given quantities of carbohydrate corresponding to the limits of their known tolerance. Stale bread was used, with a carbohydrate content roughly similar to that of the cereals. The bread was moistened with water after weighing. The cereals were weighed raw, and then boiled. No other food was given on the test days, and a fixed diet of 1 kilo of beef lung was followed on all other days. There was never glycosuria except on test days.

On test days approximately 50 gm. of starch were administered to Dog B2–43 in the following forms:

Dog B2–43.

Date.	Food.	Glycosuria.
1914		*gm.*
Sept. 10	Oatmeal.	0
" 15	Rice.	5.5
Oct. 2	Potato.	0
" 10	Bread.	6.4
" 19	Pearled barley.	2.3

The program was continued in the same animal by varying the quantities of the different foods, with a view to testing the tolerance for each.

Date.	Diet.	Glycosuria.
1915		*gm.*
Mar. 26	75 gm. oatmeal.	0
" 30	100 " "	0
Apr. 1	200 " "	0.7
" 16	50 " bread, 1 kilo lung.	Faint.
" 19	50 " " 1 " "	0
" 20	100 " " 1 " "	Slight.
" 24	100 " "	3.0
" 27	100 " oatmeal.	0
May 3	100 " "	0
" 5	150 " "	0
" 10	200 " "	Faint.
" 17	200 " " 1 kilo lung.	0
" 25	200 " rice.	8.4
June 1	100 " "	6.4
" 8	50 " "	Faint.
" 15	200 " oatmeal.	3.4
" 26	200 " rice.	1.6

Dog B2–63.

Date.	Diet.	Glycosuria.
1915		*gm.*
Apr. 16	50 gm. bread.	0
" 20–23	100 " " 1 kilo lung daily.	Traces.
" 24	150 " " 1 " "	1.6
" 27	150 " oatmeal.	0
May 3	150 " "	2.6
" 10	200 " "	2.3
" 17	200 " " 1 kilo lung.	1.1
" 25	200 " rice.	5.6
June 1	100 " "	11.7
" 8	50 " "	Trace.
" 15	200 " oatmeal.	"
" 25	200 " rice.	0
July 3	200 " bread.	0
" 6	200 " "	0

Dog B2–71.

Date.	Diet.	Glycosuria.
1915		*gm.*
Mar. 29	100 gm. bread.	0
" 30	200 " "	11.4
" 31	Fast day.	0
Apr. 1	300 gm. bread.	6.8
" 16	50 " " 1 kilo lung.	0
" 20	100 " " 1 " "	0
" 24	100 " "	1.4
" 27	100 " oatmeal.	0
May 3	100 " "	Trace.
" 5	150 " "	0
" 10	200 " "	8.7
" 17	200 " " 1 kilo lung.	9.1
" 25	200 " rice.	19.3
June 1	100 " "	18.7
" 8	50 " "	0
" 15	200 " oatmeal.	0
" 26	200 " rice.	2.2

Dog B2–76.

On test days approximately 50 gm. of starch were administered as follows:

Date.	Food.	Glycosuria.
1914		*gm.*
Sept. 10	Oatmeal.	Trace.
" 15	Rice.	3.1
Oct. 2	Potato.	1.1
" 10	Bread.	2.8
" 19	Pearled barley.	1.2

Dog B2–81.

Date.	Diet.	Glycosuria.
1915		*gm.*
Mar. 31	200 gm. bread.	0
Apr. 1	300 " "	0.73
" 16	50 " "	0
" 19	50 " " 1 kilo lung.	0
" 20	100 " " 1 " "	2.8
" 24	100 " "	0.16
" 27	100 " oatmeal.	0
May 3	100 " "	0
" 5	100 " "	0
" 10	200 " "	0
" 17	200 " " 1 kilo lung.	3.8
" 25	200 " rice.	5.3
June 1	100 " "	7.4
" 8	50 " "	0
" 15	200 " oatmeal.	Trace.

The claims for the superior assimilability of certain starchy foods, particularly oatmeal, have been previously reviewed.[7] As the claims included some observations with totally depancreatized dogs, the above tests were begun upon partially depancreatized animals, which more closely resemble human diabetics in digestive power and other respects. They were intended as orientation experiments, to decide whether an investigation with combined analyses of the food, feces, and blood sugar was worth while. The following deductions were made.

Precautions were used against fluctuations of assimilation due to changes of body weight, indigestion, diet in the intervals between test days, and other known factors. Dog B2–63 obviously gained tolerance toward the close of the experimental period; otherwise there was success in the attempt to choose animals with fairly stationary tolerance. In Dog B2–71, a single fast day on March 31 evidently increased the bread tolerance considerably, as shown by comparison of March 30 and April 1. With all care against disturbing influences, the tolerance of the animals shows occasional fluctuations from unknown causes, which would seriously hamper accurate experiments upon such test objects. The milder cases of human diabetes, which have been used for comparisons of carbohydrate tolerance, are subject to fully as great fluctuations of tolerance as these animals, and the exactness of tests upon them is open to similar question.

Owing to such accidental fluctuations, the assimilation of oatmeal might rarely seem inferior to that of rice, as in Dog B2–43 on June 15 and 26. But the number of observations was great enough to establish the rule that glycosuria from oatmeal was in general much less than that from any other carbohydrate. Generally the dogs ate the oatmeal less willingly than other foods, notably bread and rice, which caused greater glycosuria. Even when it was eaten promptly, and in the absence of diarrhea, there is the usual question whether the absorption is actually equal, and the recognized impossibility of de-

[7] Allen,[1] Chapter IX. Allen, F. M., Stillman, E., and Fitz, R., Total dietary regulation in the treatment of diabetes, Monograph of The Rockefeller Institute for Medical Research, No. 11, New York, 1919, Chapter I.

termining the rate of absorption or the extent of fermentation by fecal analyses or any other means. The above findings in dogs are similar to the clinical observations on which the oatmeal treatment was based, but the calorimetric studies of Du Bois[8] confirmed the more modern view that the assimilation of oatmeal is not actually superior to that of other starchy foods. When this more accurate method became available, the animal experiments were dropped.

Carbohydrate fed at the same time with protein in the form of beef lung sometimes caused slightly greater glycosuria than the carbohydrate alone. For example, Dog B2–71 showed slight differences of this character with oatmeal on May 10 and 17, and Dog B2–81 with bread on April 20 and 24, and with oatmeal on May 17 and June 15. But in equally numerous instances it is seen that the glycosuria was greater from carbohydrate alone (Dog B2–43, April 20 and 24, May 10 and 17; Dog B2–63, May 10 and 17; Dog B2–71, April 20 and 24), as if the mixture with protein had slowed the absorption of the carbohydrate. The observations are entirely contrary to the claim that the assimilability of oatmeal is spoiled by simultaneous ingestion of meat.

CONCLUSIONS.

1. The injurious effects of excessive carbohydrate diet are demonstrable in partially depancreatized dogs, in the same manner as in human patients. With severe diabetes there is rapid progress of emaciation and weakness and early death.

2. With milder diabetes, there is frequently a transitional state following operation, when the fate depends on the diet. If the tolerance is spared for a time, recovery sometimes occurs to such extent that diabetes cannot be produced by any kind or quantity of feeding, but only by removal of a small additional fragment of pancreatic tissue. The proper degree of carbohydrate overfeeding is important in this early period for producing the most useful type of diabetic animals; namely, those having good digestion and general health combined with a permanent lowering of assimilative power, like the condition of human patients.

[8] Allen, F. M., and Du Bois, E. F., *Arch. Int. Med.*, 1916, xvii, 1010.

3. In the early stage, glucose is more powerful than starch in producing diabetes, and animals which are progressing toward complete recovery on starch diet can be sent into hopeless diabetes by admixture of glucose. The difference seems to be merely of the rate of absorption, and indicates that a rapid flood of carbohydrate is more injurious to the pancreatic function than a slow absorption. Whenever permanent diabetes is present, so that complete recovery is impossible, starch brings on glycosuria more slowly than sugar, but just as surely. The difference in time in different cases amounts to days, weeks, or months. The clinical lesson from such experiments is that even if a patient becomes free from glycosuria on withdrawal of sugar only, nevertheless other foods should also be limited.

4. No significant differences were observed between the assimilation of different starches, or any extreme lowering of the carbohydrate tolerance by proteins, such as alleged by certain writers in connection with the "oatmeal cure."

5. Repair of traumatic inflammation and hypertrophy of the pancreas remnant have been mentioned incidentally as the basis of the early tendency to recovery, and also hydropic degeneration of Langerhans islands as an accompaniment of the lowering of tolerance by excessive diet. These are believed to have their parallels in human cases, and are to be described more fully hereafter.

STUDIES ON EXPERIMENTAL PNEUMONIA.*

I. Production of Pneumococcus Lobar Pneumonia in Monkeys.

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Plate 21.

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INTRODUCTION.

The unusual prevalence of pneumonia during the past 2 years has led to much intensive study of the disease. Perhaps the most important contributions to these studies have been the increasing emphasis laid upon etiologic classification of pneumonia, the correlation of the clinical features and pathology of the disease with the various bacteria that are capable of producing it, and the indication that prophylactic vaccination against certain types of pneumococcus pneumonia may lead to a reduction in the incidence of these types.

The different biologic types of pneumococcus, *Streptococcus hæmolyticus*, and *Bacillus influenzæ* have been the organisms most frequently associated with the pneumonia that has occurred. While it has been found possible to differentiate to a considerable extent the clinical features and pathology of the various types of pneumonia caused by these different bacteria, many points have remained obscure because of the frequent occurrence of mixed infections, especially in the pneumonia that has followed influenza. Perhaps the greatest difference in opinion has arisen with respect to the relation of *Bacillus influenzæ* to epidemic influenza and to the pneumonia which so frequently complicates it. Similarly, though the results of prophylactic vaccina-

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tion against pneumococcus pneumonia strongly suggest that a definite degree of immunity against lobar pneumonia is provided by this procedure, the interpretation of statistical studies upon the incidence of disease in man is always difficult because of uncontrollable and apparently unrelated factors that may influence the outcome of the experiment.

It seemed possible that more definite knowledge might be obtained concerning the various types of pneumonia and the efficiency of prophylactic vaccination if a study of experimental pneumonia were undertaken, since by this means pure infections might be dealt with and adequate experimental controls would be available. In addition, a study of the therapeutic efficiency of Type I antipneumococcus serum and of an outbreak of spontaneous pneumonia among the stock animals was conducted.

Since it had been previously found[1] that experimental pneumonia could readily be produced in the monkey by the intratracheal injection of small amounts of culture, this animal was selected as offering the best opportunity for success. Two species of monkeys have been used, *Macacus syrichtus* from the Philippine Islands and *Cebus capucinus* from Central America. All were fresh stock and had not previously been kept in captivity in this country. Normal animals were used throughout, no preliminary procedures to lower resistance or to produce injury to the respiratory tract being resorted to except in a few instances for special purposes as indicated in the text. The results of the study will be reported in a series of papers.[2]

[1] Opie, E. L., Freeman, A. W., Blake, F. G., Small, J. C., and Rivers, T. M., unpublished experiments.

[2] The present paper is the first of this series; Paper II follows. The others will appear in later numbers of this *Journal*. Their titles are as follows: III. Spontaneous pneumonia in monkeys. IV. Results of prophylactic vaccination against pneumococcus pneumonia in monkeys. V. Active immunity against experimental pneumococcus pneumonia in monkeys following vaccination with living cultures of pneumococcus. VI. Active immunity following experimental pneumococcus pneumonia in monkeys. VII. Treatment of experimental Pneumococcus Type I pneumonia in monkeys with Type I antipneumococcus serum. VIII. Experimental *Streptococcus hæmolyticus* pneumonia in monkeys. IX. Production of influenza in monkeys by inoculation with *Bacillus influenzæ*. X. Pathology of experimental influenza and of *Bacillus influenzæ* pneumonia in monkeys.

Pneumococcus Lobar Pneumonia.

Many attempts have been made to produce lobar pneumonia in animals.[3] In the instances in which a lobar consolidation of the lung has been successfully produced, the results have not been altogether satisfactory either because it has not been clearly shown that the animals suffered from clinical lobar pneumonia as seen in man, or because large amounts of culture material were injected under more or less artificial conditions, or because various preliminary procedures injurious to the respiratory tract were resorted to in order to render the animal susceptible to infection. In the present experiments the attempt has been made to avoid these objections in as far as was possible, while at the same time a method of inoculation was employed that would consistently produce the desired result.

Four highly virulent strains of pneumococcus have been used throughout. Three of these were stock laboratory strains originally isolated from cases of lobar pneumonia of Pneumococcus Type I, Pneumococcus Type II, and Pneumococcus Type III respectively, of which 0.0000001 cc. of an 18 hour broth culture injected intraperitoneally regularly killed white mice within 48 hours. The fourth was a pneumococcus of Type IV isolated at autopsy from a monkey dying of spontaneous lobar pneumonia. This pneumococcus killed white mice within 48 hours when injected intraperitoneally in doses of 0.000001 cc. of an 18 hour broth culture.

Methods.

The method of inoculation was by direct intratracheal injection, under aseptic precautions, by the insertion of a small caliber, dry, sterile needle into the lumen of the trachea between the tracheal cartilages just below the larynx and the introduction of the culture by means of a Luer glass syringe inserted into the stock of the needle.

[3] Wadsworth, A., *Am. J. Med. Sc.*, 1904, cxxvii, 851. (This article contains a review of the older literature.) Lamar, R. V., and Meltzer, S. J., *Proc. Soc. Exp. Biol. and Med.*, 1909–10, vii, 102; *J. Exp. Med.*, 1912, xv, 133. Wollstein, M., and Meltzer, S. J., *J. Exp. Med.*, 1912, xvi, 126. Winternitz, M. C., and Hirschfelder, A. D., *J. Exp. Med.*, 1913, xvii, 657. Sisson, W. R., and Walker, I. C., *J. Exp. Med.*, 1915, xxii, 747.

The procedure is relatively simple, takes but a few seconds, subjects the animal to a minimal amount of trauma, and is not attended by gagging or coughing. In no instance at autopsy has there been any evidence of local injury or infection in the trachea or in the tissue surrounding it. The amounts of culture injected ranged from 0.000001 to 1 cc. of an 18 hour plain broth culture. A constant amount of fluid, 1 cc., was used throughout, dilutions of cultures being made in plain broth.

The methods of study after inoculation consisted in observation and record of clinical symptoms and physical signs, the taking of morning and afternoon temperature (rectal), daily counts of the white blood corpuscles with examination of stained blood films and differential white blood counts, and daily blood cultures in broth and in poured agar plates with record of the number of pneumococcus colonies developing per 0.5 cc. of blood. No record of the pulse or respiration rate was kept, since it was not found feasible to record these accurately. X-ray plates were taken in a few instances (Figs. 1 and 2), but no systematic study was made. Autopsies with bacteriological examination were performed on all animals that died or were killed.

EXPERIMENTAL.

Production of Lobar Pneumonia by the Intratracheal Injection of Pneumococcus.

Thirty-seven normal monkeys were injected intratracheally with cultures of pneumococcus in amounts ranging from 1 to 0.000001 cc. of an 18 hour broth culture, a constant amount of fluid, 1 cc., being used in all the experiments. Pneumococcus Type I was used thirty-one times; Pneumococcus Type II, twice; Pneumococcus Type III, three times; and Pneumococcus Type IV, once. In thirty-two instances lobar pneumonia in all its aspects resembling the disease as seen in man was successfully produced. In five cases the monkeys failed to develop pneumonia. The results are shown in Table I.

It will be seen from the data presented in Table I that pneumonia was as readily produced by the injection of 0.000001 cc. of pneumococcus as with larger amounts. Of the twenty-six monkeys in which

lobar pneumonia was produced with Pneumococcus Type I, twenty-one died, and five recovered, the latter all receiving amounts of less than 0.001 cc. With the exception of the fact that all the monkeys which received 0.001 cc. of culture or more died, there is no evident relation between the duration of the disease and final outcome on the one hand and the weight of the monkey or amount of culture injected on the other. The differences probably depended upon individual variation in resistance to pneumococcus infection.

Although the strains of Pneumococcus Type II, Type III, and Type IV used were as virulent for mice as the strain of Pneumococcus Type I, they proved much less so for monkeys. Of the six monkeys injected with these organisms all recovered, although a relatively large amount of culture, 0.1 to 1 cc., was used.

No attempt is made to explain the failure to produce pneumonia in five animals. Monkey 4 died in 23½ hours with an overwhelming septicemia. Monkey 27 died on the 3rd day with an acute fibrinopurulent pneumococcus pericarditis and overwhelming septicemia. Monkeys 63, 76, and 128, all of which received minute amounts of culture, showed no evidence of infection.

In addition to the cases shown in Table I, lobar pneumonia was successfully produced in a similar manner in twenty-nine other experiments, twenty times with Pneumococcus Type I, twice with Pneumococcus Type II, twice with Pneumococcus Type III, and five times with Pneumococcus Type IV. These monkeys had either been vaccinated previously with pneumococcus vaccine, or had had a preceding attack of pneumonia, or were treated with antipneumococcus serum, and therefore, although they were in normal health at the time of injection, they are excluded from the series of normal animals shown in Table I. Experiments dealing with them will be presented in Papers IV to VII. The clinical records of some, however, are included in this paper in order to illustrate certain features of the disease.

In the following paragraphs abbreviated protocols illustrative of the clinical features of the pneumonia experimentally produced in monkeys are presented, study of the pathology of the disease being reserved for Paper II. Since many of the experiments were entirely similar, it has not seemed necessary to give the protocols of all, those

TABLE I.

Effect of Intratracheal Inoculation of Monkeys with Pneumococcus.

Monkey No.	Species.	Weight.	Date of inoculation.	Type of pneumococcus.	Amount of culture.	Clinical diagnosis.	Result.	Autopsy.
		gm.	*1919*		*cc.*			
1	*C. capucinus.*	1,746	Feb. 17	I	1.0	L. P.*	Died on 2nd day.	Lobar pneumonia, R. L.;† engorgement and red hepatization.
2	" "	1,290	" 24	I	0.5	"	" in 12½ hrs.	Lobar pneumonia, R. U., R. L., L. U., L. L.; engorgement and red hepatization.
3	" "	1,595	" 24	I	0.1	"	" on 2nd day.	Lobar pneumonia, L. U., L. L.; engorgement and red hepatization.
4	" "	1,835	" 26	I	0.01	?	" in 23½ hrs.	No pneumonia. Pneumococcus septicemia.
5	" "	1,410	Mar. 5	I	0.01	L. P.	" " 23¼ hrs.	Lobar pneumonia, R. U., R. L., L. L.; engorgement and red hepatization.
6	" "	1,960	" 5	I	0.001	"	" on 4th day.	Lobar pneumonia, R. U., R. L.; engorgement and red hepatization; acute fibrinous pleuritis, right.
23	*M. syrichtus.*	3,555	" 18	I	1.0	"	" " 5th "	Lobar pneumonia, L. L., engorgement and red hepatization; R. L., engorgement; acute fibrinous pleuritis, left.
26	" "	2,680	" 21	I	1.0	"	Dying, killed 9th day.	Lobar pneumonia, R. L., L. L.; gray hepatization; acute fibrinous pleuritis, right and left.
27	" "	2,620	" 26	I	1.0	P. S.	Died on 3rd day.	Acute fibrinopurulent pericarditis; pneumococcus septicemia; no pneumonia.
29	" "	3,920	Apr. 2	I	1.0	L. P.	" " 2nd "	Lobar pneumonia, L. U., L. M., L. L.; engorgement and red hepatization; acute fibrinous pleuritis, left.

30	*M. syrichtus.*	4,120	Apr. 2	I	0.1	L. P.	Died on 12th day.	Lobar pneumonia, R. U., R. M., R. L., L. U., L. M., L. L.; gray hepatization and beginning resolution; acute fibrinous pleuritis, right and left; dilatation of heart.
31	" "	3,850	" 2	I	0.01	"	" " 10th "	Lobar pneumonia, L. U., L. M., L. L.; gray hepatization; empyema, left; acute purulent mediastinitis.
25	*C. capucinus.*	740	" 4	II	0.1	"	Recovered by crisis on 7th day. Killed.	Lobar pneumonia, R. U.; gray hepatization and beginning resolution; acute fibrinous pleuritis, right.
42	*M. syrichtus.*	4,955	" 10	I	0.001	"	Died on 37th day.	Lobar pneumonia, L. M., L. L., organization; R. U., R. M., R. L., partial resolution and organization; chronic fibrous pleuritis, right and left.
63	" "	2,100	" 19	I	0.000001	‡	Remained well.	No autopsy.
75	" "	4,200	" 29	I	0.0001	L. P.	Recovered by crisis on 12th day.	" "
76	" "	4,600	" 29	I	0.00001	‡	Remained well.	" "
77	" "	2,600	" 29	I	0.000001	L. P.	Recovered by crisis on 11th day.	" "
82	*C. capucinus.*	730	" 30	III	0.1	"	Recovered by crisis on 7th day. Killed.	Interstitial pneumonia, R. U.; resolving.
83	*M. syrichtus.*	2,470	" 30	I	0.001	"	Died on 8th day.	Lobar pneumonia, R. U., R. M., R. L.; red and gray hepatization; acute serofibrinous pleuritis, right.
85	" "	2,675	May 6	I	0.001	"	" " 6th "	Lobar pneumonia, L. M., L. L.; engorgement and red hepatization; acute fibrinous pleuritis, left.

* L. P indicates lobar pneumonia; P. S., pneumococcus septicemia.

† R. L., R. M., R. U., etc., indicate lobes of the lung. The cardiac lobe is included as part of the right lower lobe.

‡ No disease.

TABLE I—*Concluded.*

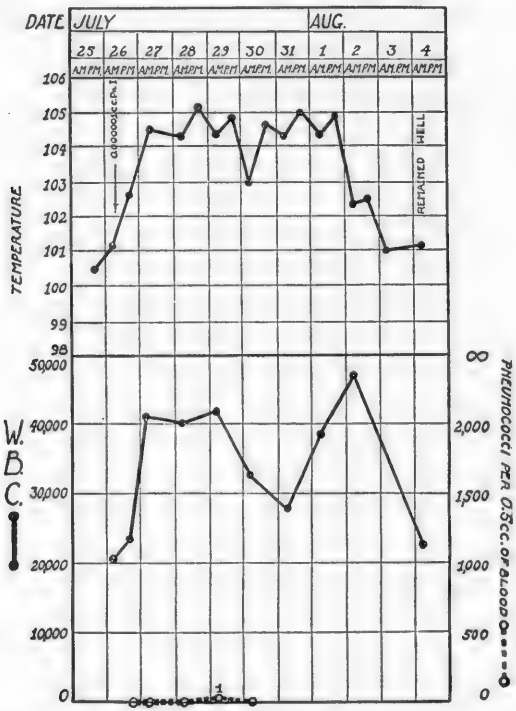
Monkey No.	Species.	Weight.	Date of inoculation.	Type of pneumococcus.	Amount of culture.	Clinical diagnosis.	Result.	Autopsy.
		gm.	*1919*		*cc.*			
86	*M. syrichtus.*	2,424	May 6	I	0.000001	L. P.	Died on 14th day.	Lobar pneumonia, R. L., L. L.; gray hepatization; acute fibrinous pleuritis, right and left.
87	" "	2,835	" 6	I	0.1	"	" " 5th "	Lobar pneumonia, L. U., L. M., L. L., red hepatization; R. L., engorgement; acute fibrinous pleuritis, left.
95	" "	4,100	" 13	I	0.00001	"	Recovered by crisis on 11th day. Relapse (?) on 15th day. Recovery by crisis on 21st day. Killed.	Lobar pneumonia, L. L.; partial resolution and organization; organizing pleuritis, left.
96	" "	2,655	" 13	I	0.000001	"	Recovered by crisis on 9th day.	No autopsy.
93	" "	5,110	" 15	I	0.001	"	Died on 13th day.	Lobar pneumonia, R. U., R. M., R. L.; gray hepatization; acute fibrinous pleuritis, right; hypertrophy and dilatation of heart.
98	" "	4,100	" 20	I	0.000001	"	" " 4th "	Lobar pneumonia, R. U., R. L., L. U., L. L.; engorgement.
109	" "	4,585	" 27	I	0.001	"	" " 3rd "	Lobar pneumonia, R. U., R. M., L. U., L. M.; engorgement.
110	" "	2,710	" 27	I	0.000001	"	" " 7th "	Lobar pneumonia, R. U., R. M., R. L.; red and gray hepatization; acute fibrinopurulent pericarditis; acute fibrinous pleuritis, right.

111	*M. syrichtus.*	2,220	June 27	IV	0.1	L. P.	Recovered by crisis on 11th day.	No autopsy.
112	" "	3,975	" 5	I	0.01	"	Died on 6th day.	Lobar pneumonia, R. M., R. L., red and gray hepatization; L. L., engorgement; acute fibrinous pleuritis, right.
114	" "	3,000	" 20	I	0.000001	"	" " 5th "	Lobar pneumonia, R. L., L. L.; engorgement.
91	" "	4,005	" 24	II	0.1	"	Recovered by crisis on 18th day.	No autopsy.
115	" "	3,015	" 24	III	0.1	"§	Recovered by lysis on 3rd day.	" "
107	" "	2,335	July 1	III	1.0	"§	Recovered by lysis on 4th day. Killed.	Interstitial pneumonia, R. L.; acute fibrinous pleuritis, right.
127	" "	2,700	" 24	I	0.000001	"	Recovered by crisis on 8th day.	No autopsy.
128	" "	2,650	" 26	I	0.000001	‡	Remained well.	" "

§ Abortive.

presented having been selected with the purpose of bringing out as fully as possible the various aspects of the disease. The temperature, white blood counts, and results of blood cultures are shown in Text-figs. 1 to 17.

Experiment 1. Monkey 115 (Text-Fig. 1).—Macacus syrichtus, female; weight 3,015 gm. Previously inoculated with Pneumococcus Type III on June 24, and



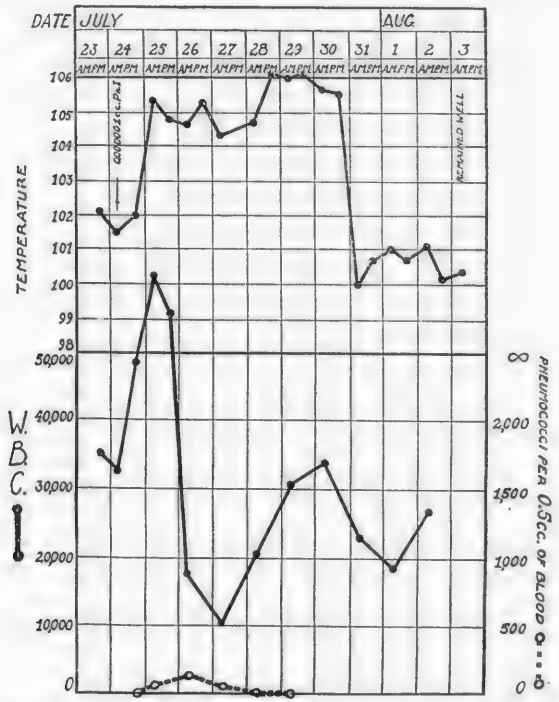
TEXT-FIG. 1. Monkey 115. Experimental lobar pneumonia following the intratracheal injection of 0.000001 cc. of Pneumococcus Type I broth culture.

with Pneumococcus Type IV on July 1, 1919. July 25. Well and active. July 26, 10.20 a.m. Intratracheal injection of 0.000001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. 4 p.m. Appears well. July 27. Moderately sick; respirations rapid and labored, with expiratory grunt. July 28. Condition the same. July 29. Quite sick; no appetite; breathing rapidly; coughs frequently.

Dullness and suppressed breath sounds over right lower lobe. July 30 to Aug. 1. Condition the same. Aug. 2. Appears well and active; breathing easily; temperature fallen to normal by crisis. Aug. 4. Continues well.

Diagnosis.—Lobar pneumonia, right lower lobe.

Experiment 2. Monkey 117 (Text-Fig. 2).—*Macacus syrichtus*, male; weight 2,565 gm. July 23, 1919. Well and active. July 24, 11 a.m. Intratracheal injection of 0.000001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type

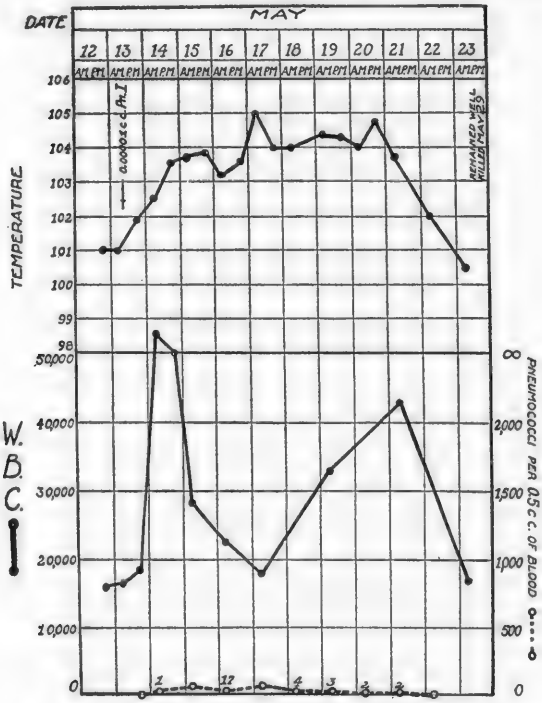


TEXT-FIG. 2. Monkey 117. Experimental lobar pneumonia following the intratracheal injection of 0.000001 cc. of Pneumococcus Type I broth culture.

I. 4.30 p.m. Appears well. July 25, 11 a.m. Shivering violently. Respirations accelerated. July 26. Sick; respirations rapid and labored; lungs clear. July 27. Condition the same. July 28. Refuses food; breathing rapidly; dullness over right lower lobe. July 29. Condition the same. Marked dullness, suppressed breathing, and fine moist râles over right lower lobe; coughs occasionally. July 30. Condition the same. July 31. Appears well; breathing easily; temperature fallen to normal by crisis. Aug. 5. Continues well.

Diagnosis.—Lobar pneumonia, right lower lobe.

Experiment 3. Monkey 81 (Text-Fig. 3).—Macacus syrichtus, female; weight 2,750 gm. May 12, 1919. Well and active. May 13, 10.55 a.m. Intratracheal injection of 0.00001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. 4.20 p.m. Appears well. May 14. Quiet but otherwise appears well. May 15. Moderately sick; breathing rapidly. May 16 to 18. Condition the same. May 19 to 22. Appears sicker; huddled up leaning against side of cage; respira-



TEXT-FIG. 3. Monkey 81. Experimental lobar pneumonia following the intratracheal injection of 0.00001 cc. of Pneumococcus Type I broth culture.

tions rapid and labored. May 23. Appears much better, but manifests considerable weakness and dyspnea on exertion; temperature normal. May 25. Appears stronger. May 29. Remains well. Killed.

Autopsy.—Lobar pneumonia, right middle and lower lobes, resolving; acute fibrinous pleuritis, right.

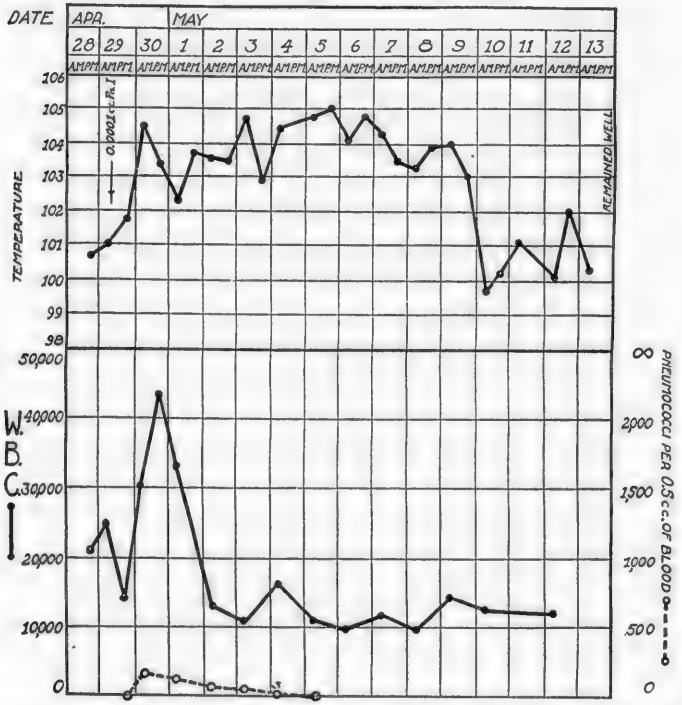
Cultures.—Heart's blood and right lower lobe, no growth.

Monkeys 115, 117, and 81 show well the characteristic clinical features of relatively mild lobar pneumonia with recovery. It will be noted that the temperature curves are identical with those of similar cases in man, presenting the features of sudden onset, sustained elevation throughout the course of the disease, and a critical fall to normal on the 7th or 8th day. The characteristic chill at onset was observed in Monkey 117. In relatively mild pneumonia such as these cases represent monkeys appear only moderately sick, sitting up throughout the course of the disease, and never exhibiting any degree of prostration. The clinical symptoms consist of characteristic rapid and labored respiration, often with expiratory grunt, infrequent cough rarely appearing before the latter half of the disease, and loss of appetite and activity. Monkeys 115 and 117 show a characteristic transient invasion of the blood by pneumococci with disappearance of the bacteremia several days before crisis. Monkey 81 shows a low grade bacteremia throughout the active stage of the disease, an uncommon feature in monkeys that recovered. The characteristic leucocyte curve in non-fatal pneumonia is well illustrated in all three cases. It consists of a preliminary high polymorphonuclear leucocytosis followed by a fall to normal, a secondary rise during the latter half of the disease, and a return to normal with crisis. It will be noted that the first fall of leucocytes to normal is approximately coincident with the peak of the bacteremia, a feature that has occurred with surprising constancy throughout the series of experiments. This type of leucocyte reaction has occurred in all monkeys that have recovered, with the exception of four in which the secondary rise was not demonstrated.

Experiment 4. Monkey 75 (Text-Fig. 4).—Macacus syrichtus, male; weight 4,200 gm. Apr. 28, 1919. Well and active. Apr. 29, 11.10 a.m. Intratracheal injection of 0.0001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. 5.10 p.m. Appears well. Apr. 30, 11 a.m. Sits huddled up, shivering; respirations slightly accelerated. May 1. Sick; refuses food; respirations rapid with expiratory grunt. Moderate dullness, diminished breath sounds, and fine moist râles in left lower axilla. May 2. Condition the same. Dullness and bronchial breathing over left lower lobe. May 3 to 8. Condition remains the same; coughs frequently. May 9. Improving. May 10. Seems well and active, though weak. Temperature fallen to normal by crisis. May 15. Remains well. X-ray photograph of chest shows an area of density in the left lower chest (Fig. 1).

Diagnosis.—Lobar pneumonia, left lower lobe.

Monkey 75 illustrates a prolonged severe case of lobar pneumonia with final recovery by crisis on the 12th day. The sudden onset with chill, the rapid respiration with expiratory grunt, and the physical signs are all characteristic of typical lobar pneumonia. The diagnosis was confirmed by x-ray. The absence of a secondary rise in the



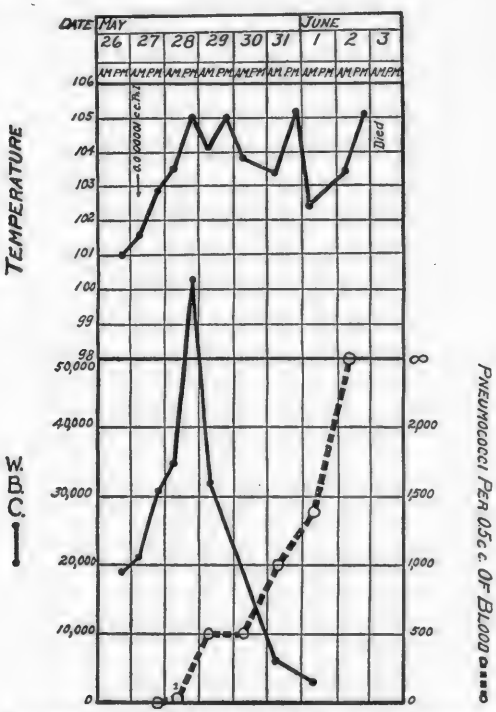
TEXT-FIG. 4. Monkey 75. Experimental lobar pneumonia following the intratracheal injection of 0.0001 cc. of Pneumococcus Type I broth culture.

leucocyte curve is illustrated in this case, a feature of unusual occurrence in monkeys that recovered.

Experiment 5. Monkey 110 (*Text-Fig. 5*).—*Macacus syrichtus*, female; weight 2,710 gm. May 26, 1919. Well and active. May 27, 10.20 a.m. Intratracheal injection of 0.000001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. May 28. Quiet; respirations rapid. May 29. Appears moderately

sick; refuses food; breathing rapidly. May 30. Condition the same. May 31 to June 1. Very sick; respirations rapid and labored; sits huddled up in corner of cage. June 2. Very sick; respirations rapid and gasping; lies down on floor of cage at times. June 3. Found dead in morning.

Autopsy.—Lobar pneumonia, right upper, middle, and lower lobes; red and gray hepatization; acute fibrinopurulent pericarditis; acute fibrinous pleuritis, right; cardiac dilatation.

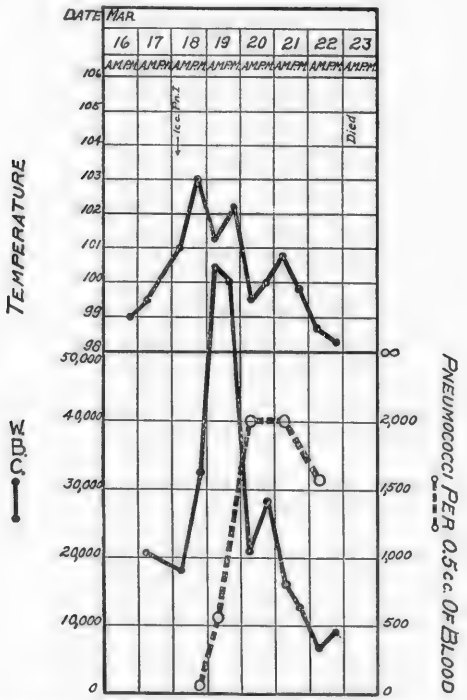


TEXT-FIG. 5. Monkey 110. Experimental lobar pneumonia following the intratracheal injection of 0.000001 cc. of Pneumococcus Type I broth culture.

Cultures.—Heart's blood, bronchus, right lower lobe, and pericardial fluid, Pneumococcus Type I.

Experiment 6. Monkey 23 (Text-Fig. 6).—Macacus syrichtus, male; weight 3,555 gm. Mar. 17, 1919. Well and active. Mar. 18, 10 a.m. Intratracheal injection of 1 cc. of 18 hour broth culture of Pneumococcus Type I. Mar. 19, 10 a.m. Appears more quiet than usual, but appetite is good and animal moves about

2 p.m. Appears sick; breathing rapidly; lungs clear. X-ray of chest shows slightly increased density in the left mid-lung area. Mar. 20. Sick; respirations rapid and grunting; refuses food; abdomen distended and tense. Moderate dullness and distant breath sounds in left lower axilla. Mar. 21. Very sick; respirations rapid and labored. Examination of chest shows pleural friction rub, dullness, and faint bronchial breathing in left lower axilla. Mar. 22. Condition the same. Mar. 23. Found dead in morning.



TEXT-FIG. 6. Monkey 23. Experimental lobar pneumonia following the intratracheal injection of 1 cc. of Pneumococcus Type I broth culture.

Autopsy.—Lobar pneumonia, left lower lobe; engorgement and red hepatization; incipient lobar pneumonia, right lower lobe; acute fibrinous pleuritis, left.

Cultures.—Heart's blood and left lower lobe, Pneumococcus Type I.

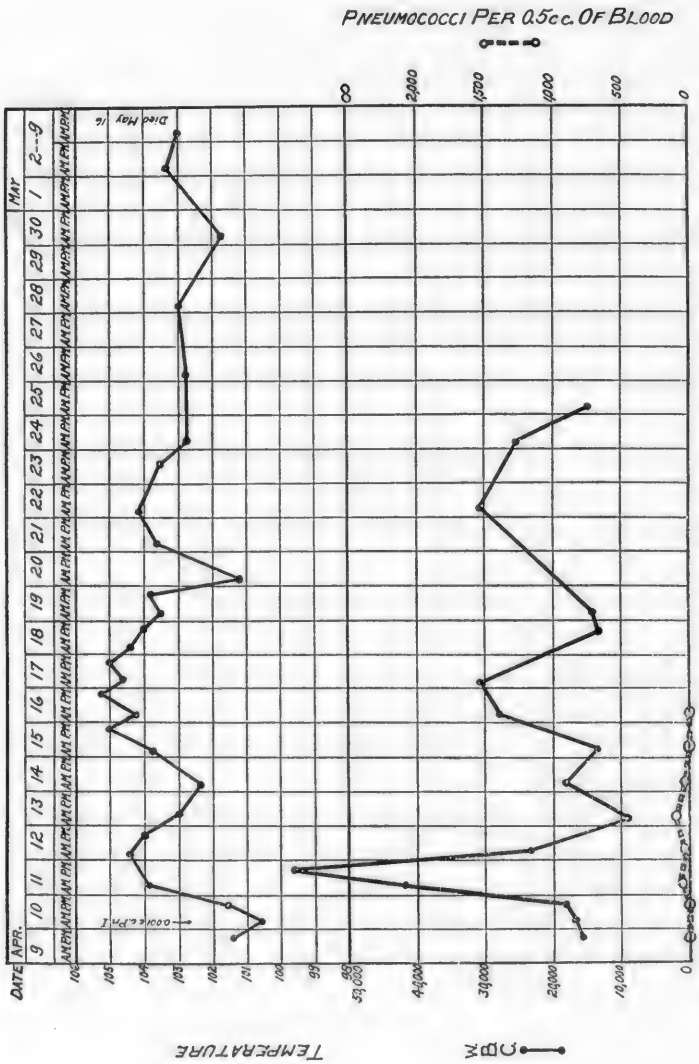
Monkeys 110 and 23 illustrate severe fatal cases of lobar pneumonia with overwhelming pneumococcus septicemia, Monkey 110

dying on the 7th day with complete consolidation of the right lung and a complicating pneumococcus pericarditis, and Monkey 23 dying on the 5th day with a moderately advanced lesion of the left lower lobe. The continued fall of the leucocyte curve with the development of a definite leucopenia coincident with the increasing septicemia has consistently occurred in cases of this nature. During this continued fall in leucocytes examination of stained blood films has shown increasing replacement of mature polymorphonuclear leucocytes by young forms until the former have practically disappeared from the blood. This has been accompanied in some instances by a terminal invasion of the blood by a few nucleated red blood corpuscles and other abnormal cells of the leucocyte series. It is noteworthy that even in the most severe cases of this kind monkeys show little evidence of prostration and maintain the sitting posture until within a relatively short time before death. Monkey 23 shows the unusual feature of a poor febrile reaction, an occurrence that is occasionally seen in rapidly fatal lobar pneumonia in man.

Experiment 7. Monkey 42 (Text-Fig. 7).—Macacus syrichtus, male; weight 4,955 gm. Apr. 9, 1919. Well and active. Apr. 10, 11 a.m. Intratracheal injection of 0.001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. Apr. 11. Appears well. Apr. 12. Moderately sick. Apr. 13. Sick; sits huddled up; breathing rapidly. Apr. 14. Condition the same. Dullness, suppressed breathing, fine moist râles, and pleural friction rub in right lower axilla. Apr. 16. Respirations rapid and labored; coughs occasionally. Dullness, bronchial breathing, and moist râles throughout both axillæ. Apr. 17 to 19. Condition the same. Apr. 20. Appears much better; temperature has fallen, but animal is still breathing rapidly. Apr. 22. Fails to improve. Temperature elevated. Apr. 28. No improvement. Continues to run an irregular fever. Slight exertion causes marked dyspnea. May 9. Animal growing progressively weaker; exhibits constant dyspnea which is greatly increased on exertion. Heart sounds loud and thumping; no murmurs. Dullness and loud bronchial breathing persist over left lower lobe; to and fro crackling râles are heard, probably pleural in origin. No evidence of empyema or pericarditis. May 10 to 15. Condition remains unchanged. May 16. Found lying on floor of cage in state of collapse. Respirations shallow and gasping. 11 a.m. Died.

Autopsy.—Lobar pneumonia, left lower and middle lobes, unresolved; right upper, middle, and lower lobes, nearly resolved; chronic fibrous pleuritis, left and right.

Cultures.—Heart's blood, bronchus, and left lower lobe, Pneumococcus Type I.



TEXT-FIG. 7. Monkey 42. Experimental lobar pneumonia, unresolved, following the intratracheal injection of 0.001 cc. of Pneumococcus Type I broth culture.

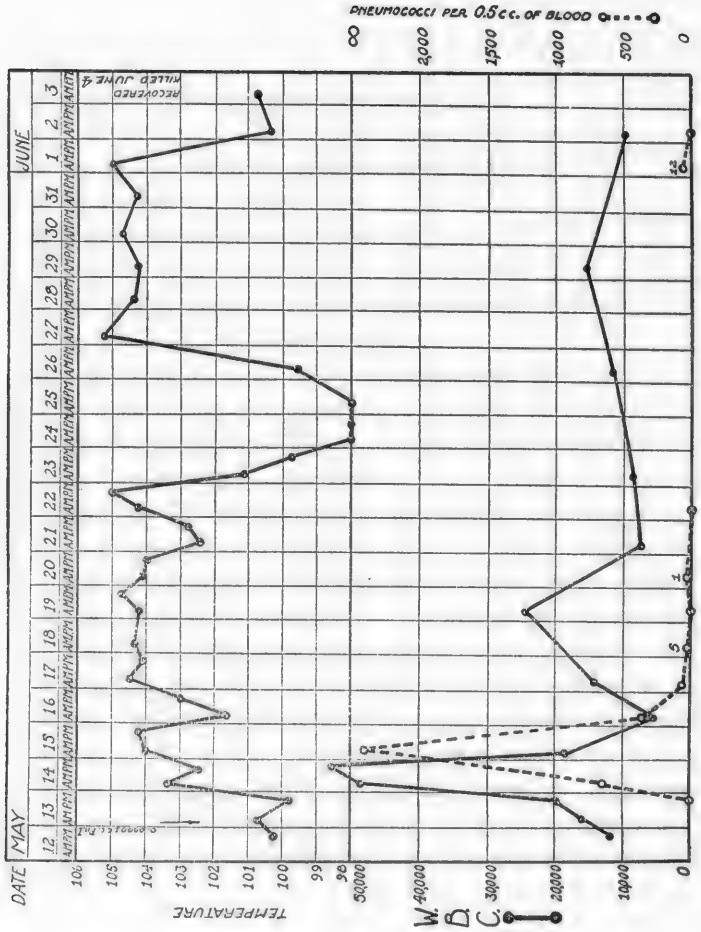
Monkey 42 illustrates a prolonged course due to an unresolved and organizing pneumonia with final death on the 37th day. The characteristic temperature curve with critical fall on the 11th day, and characteristic leucocyte reaction with fall to normal coincident with the peak of the septicemia and secondary rise are well shown. Following apparent recovery from the acute stage of the disease the monkey ran a continuous irregular temperature, manifested extreme weakness and constant shortness of breath on slight exertion, and showed persisting physical signs of consolidation in the left lower lobe. An instructive feature of the autopsy was the finding of pneumococci still present in the unresolved left lower lobe and in the heart's blood, the latter probably being a terminal invasion.

Experiment 8. Monkey 95 (Text-Fig. 8).—Macacus syrichtus, male; weight 4,100 gm. May 12, 1919. Well and active. May 13, 11.05 a.m. Intratracheal injection of 0.00001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. May 14. Quiet; respirations moderately accelerated. May 15 and 16. Appears sick; respirations rapid and grunting. May 17. Dullness and suppressed breath sounds over right lower lobe. May 18 to 22. Sick; respirations rapid and labored. May 23. Temperature fallen by crisis; appears much better. May 26. In good condition. May 27. Quiet; temperature elevated. May 28. Appears sick; huddled up in corner of cage; breathing rapidly. May 31. Condition remains the same. No evidence of pericarditis or empyema. June 2. Temperature fallen by crisis; appears better. June 4. Continues well. Killed.

Autopsy.—Lobar pneumonia, left lower lobe; pleuritis, left.

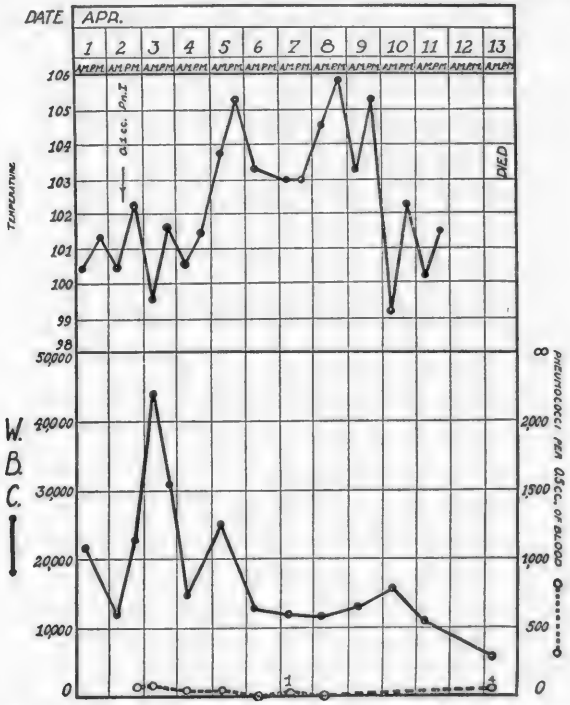
Cultures.—Heart's blood and left lower lobe, no growth.

Monkey 95 presents two unusual features. The first is that of recovery in spite of a very heavy early invasion of the blood stream by pneumococci. This has occurred in few instances. The second is an apparent relapse with a second attack of pneumonia beginning on the 4th day after recovery from the first attack. Examination of the chest during the first attack showed evidence of consolidation in the right lower lobe. It is possible, however, that this was a mistaken diagnosis since autopsy showed only a partially resolved pneumonia of the left lower lobe. There was no evidence of any complication. That the second attack was due to Pneumococcus Type I is shown by the isolation of that organism from the blood on June 1.



TEXT-FIG. 8. Monkey 95. Experimental lobar pneumonia, with relapse, following the intratracheal injection of 0.00001 cc. of Pneumococcus Type I broth culture.

Experiment 9. Monkey 30 (Text-Fig. 9).—Macacus syrichtus, male; weight 4,120 gm. Apr. 1, 1919. Well and active. Apr. 2, 11.10 a.m. Intratracheal injection of 0.1 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. Apr. 3. Appears well. Apr. 4. Moderately sick; breathing rapidly; breath sounds suppressed over left lower lobe. Apr. 5 to 7. Condition the same. Apr. 8. Sicker; respirations rapid and grunting. Examination of chest shows



TEXT-FIG. 9. Monkey 30. Experimental lobar pneumonia following the intratracheal injection of 0.1 cc. of Pneumococcus Type I broth culture. Death from cardiac dilatation (?).

dullness and loud bronchial breathing throughout left axilla, pleural friction rub, moderate dullness, and distant bronchial breathing in right lower front and axilla. Apr. 10. Appears better, but still weak and breathing rapidly. Temperature fallen by crisis. Apr. 11 and 12. Condition the same. Apr. 13, 11.30 p.m. Sudden collapse and death.

Autopsy.—Lobar pneumonia, all lobes; gray hepatization; acute fibrinous pleuritis, bilateral; cardiac hypertrophy and marked dilatation of right auricle and ventricle.

Cultures.—Heart's blood and lungs, no growth.

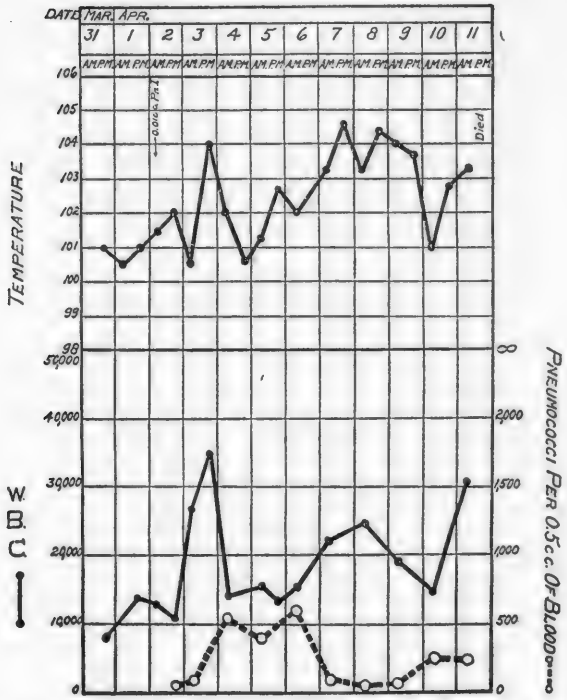
Monkey 30 likewise illustrates two unusual features. The first is an apparent delay in onset of the disease as manifested by symptoms and temperature reaction. It is noteworthy, however, that pneumococci had invaded the blood within 6 hours after intratracheal injection and that the preliminary leucocytosis was completed before there was clinical evidence that the monkey had developed pneumonia. The second is apparent recovery with crisis on the 8th day after inoculation, but death on the 12th day, autopsy revealing extensive consolidation involving all the lobes and marked dilatation of the heart. Cultures from the lungs and heart's blood remained sterile.

Experiment 10. Monkey 31 (Text-Fig. 10).—Macacus syrichtus, female; weight 3,850 gm. Apr. 1, 1919. Well and active. Apr. 2, 11.45 a.m. Intratracheal injection of 0.01 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. Apr. 3. Appears quiet but otherwise well. Apr. 4. Moderately sick; no appetite; respirations rapid. Apr. 6. Sicker; respirations very rapid and grunting. Apr. 8. Condition the same. Dullness, fine moist râles, and bronchial breathing over left lower and middle lobes. Apr. 9. Coughs occasionally. Apr. 10. Condition the same; pleural friction rub in left axilla. X-ray shows a dense shadow in the fourth and fifth left interspaces. Apr. 11. Very sick; respirations deep and gasping. 3 p.m. Died.

Autopsy.—Lobar pneumonia, left upper, middle, and lower lobes; gray hepatization; localized empyema, left; acute purulent mediastinitis.

Cultures.—Heart's blood, bronchus, and empyema, Pneumococcus Type I; left lower lobe, no growth.

Monkey 31 represents lobar pneumonia complicated by empyema. The inverse relation between the degree of leucocytosis and the degree of septicemia during the acute stage of the disease is quite striking. A persistent septicemia with rising leucocyte count during the latter part of the disease as shown in this case has nearly always been associated with the development of a complication such as empyema or pericarditis.



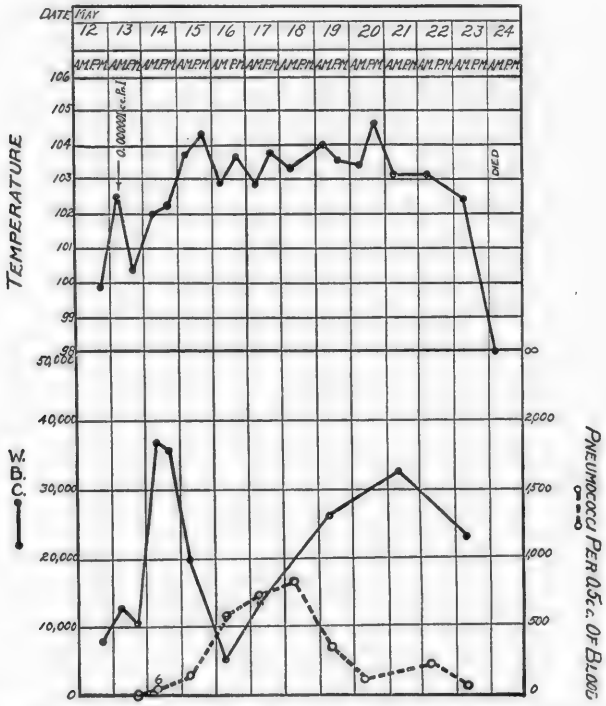
TEXT FIG. 10. Monkey 31. Experimental lobar pneumonia complicated by empyema following the intratracheal injection of 0.01 cc. of Pneumococcus Type I broth culture.

Experiment 11. Monkey 80 (Text-Fig. 11).—Macacus syrichtus, female; weight 2,850 gm. May 12, 1919. Well and active. May 13, 10.35 a.m. Intratracheal injection of 0.000001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. May 14. Quiet but otherwise appears well. May 15. Sick; respirations rapid and labored. May 16. Condition the same. May 17. Very sick; breathing rapidly. Abdomen distended and tympanitic. Dullness and diminished breath sounds in left lower axilla. May 18 to 22. Condition the same. May 23. Appears better. May 24. Very sick; lying on floor of cage; respirations rapid and gasping. 11.20 a.m. Died.

Autopsy.—Lobar pneumonia, left lower and middle lobes; gray hepatization; acute fibrinopurulent pericarditis; acute fibrinous pleuritis, left.

Cultures.—Heart's blood, pericardial fluid, left lower lobe, Pneumococcus Type I.

Monkey 80 represents a case of lobar pneumonia complicated by a pneumococcus pericarditis. The persisting septicemia with increasing leucocytosis during the latter part of the disease is well shown as in the case of Monkey 31.

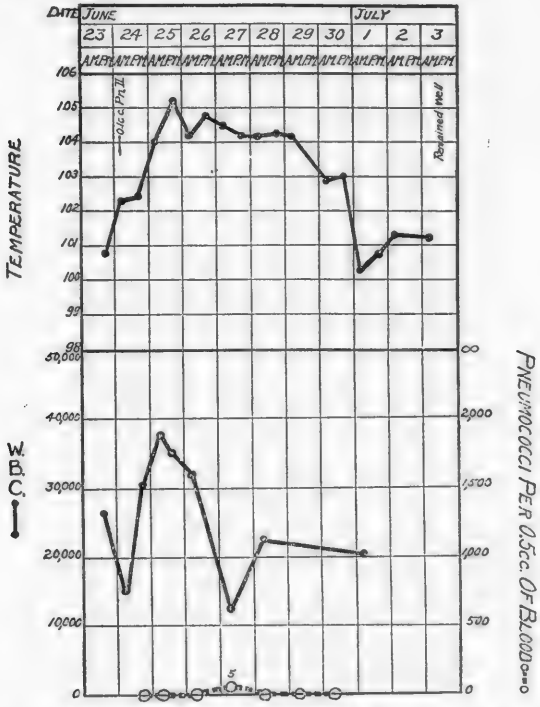


TEXT-FIG. 11. Monkey 80. Experimental lobar pneumonia complicated by fibrinopurulent pericarditis following the intratracheal injection of 0.000001 cc. of Pneumococcus Type I broth culture.

Experiment 12. Monkey 48 (Text-Fig. 12).—Macacus syrichtus, male; weight 3,590 gm. June 23, 1919. Well and active. June 24, 9.55 a.m. Intratracheal injection of 0.1 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type II. June 25. Quiet; breathing moderately accelerated. June 26. Sick; refuses food; breathing rapidly; coughs occasionally. June 27 to 29. Condition the same. June 30. Appears better; more active. July 1. Temperature fallen by crisis. Appears well and active. July 9. Continues well.

Diagnosis.—Lobar pneumonia.

Monkey 48 presents the typical picture of a mild lobar pneumonia caused by Pneumococcus Type II and is analogous in all respects to similar cases produced by the intratracheal injection of Pneumococcus Type I.

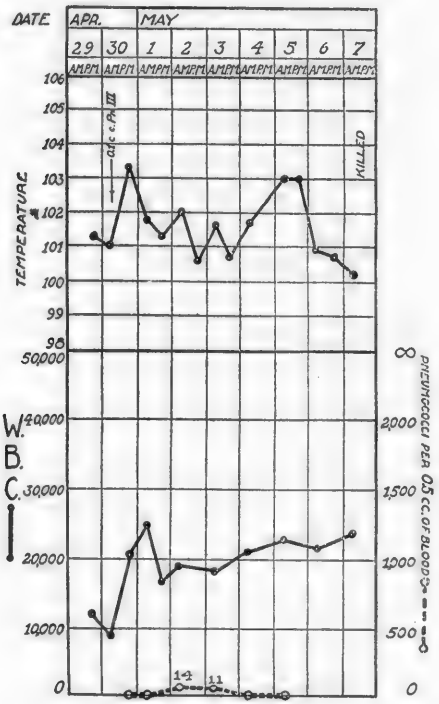


TEXT-FIG. 12. Monkey 48. Experimental lobar pneumonia following the intratracheal injection of 0.1 cc. of Pneumococcus Type II broth culture.

Experiment 13. Monkey 82 (*Text-Fig. 13*).—*Cebus capucinus*, male; weight 730 gm. Apr. 29, 1919. Well and active. Apr. 30, 10.15 a.m. Intratracheal injection of 0.1 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type III. 4.20 p.m. Appears sick; shivering violently; breathing rapidly. May 1. Sick; sitting quietly in corner of cage. Respirations rapid; abdomen distended; fine moist râles heard in right axilla. May 2 and 3. Condition the same. Moderate dullness, moist râles, and distant bronchial breathing over right upper lobe. X-ray shows moderately increased density in the right upper lobe. May 5. Improving. May 7. Appears well and active. Killed.

Autopsy.—Resolving interstitial pneumonia, right upper lobe.
Cultures.—Heart's blood and trachea, no growth.

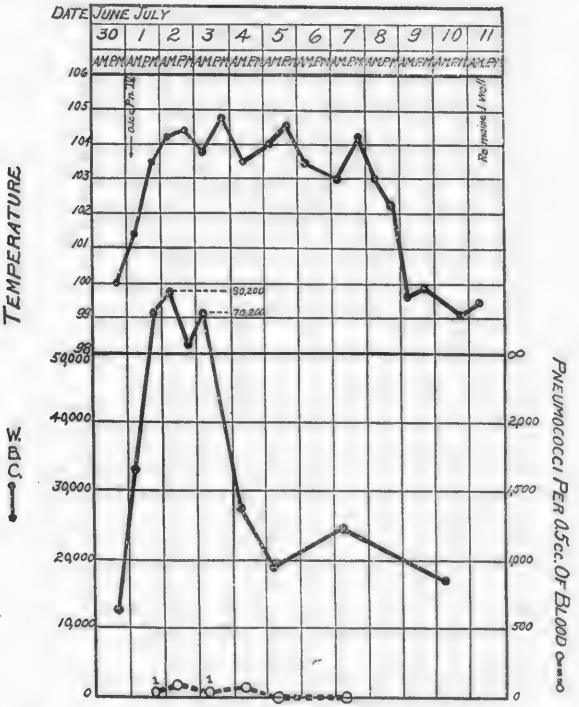
Monkey 82 represents a mild pneumonia due to Pneumococcus Type III. The onset was abrupt, with characteristic chill and sharp elevation in temperature and leucocyte count. The temperature curve, however, was irregular and unusual. It has been stated that



TEXT-FIG. 13. Monkey 82. Experimental pneumonia following the intratracheal injection of 0.1 cc. of Pneumococcus Type III broth culture.

the strain of Pneumococcus Type III used was relatively avirulent for monkeys. This is clearly shown in Monkeys 115 and 107 (Table I) both of which ran short courses of 3 and 4 days duration without invasion of the blood by the pneumococcus. It is probable that similar cases which are occasionally seen in man would be diagnosed as

bronchopneumonia or abortive lobar pneumonia. The latter term would seem preferable, since histological examination has shown that invasion of the lung in these monkeys is identical with the mode of invasion in experimental lobar pneumonia, but that further extension of the process to the stage of alveolar exudation and complete lobar consolidation is checked, presumably because of the resistance of the animal against infection with this strain of pneumococcus. This point is discussed further in Paper II.



TEXT-FIG. 14. Monkey 115. Experimental lobar pneumonia following the intratracheal injection of 0.1 cc. of Pneumococcus Type IV broth culture.

Experiment 14. Monkey 115 (Text-Fig. 14).—Macacus syrichtus, female; weight 3,015 gm. Previously inoculated with Pneumococcus Type III on June 24, 1919. June 30. Well and active. July 1, 11 a.m. Intratracheal injection of 0.1 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type IV. July 2.

Moderately sick; breathing rapidly. July 3. Sick; no appetite; respirations labored and rapid. July 4 to 8. Condition the same. Coughs occasionally. July 9. Appears well and active. Temperature fallen to normal by crisis. July 16. Continues well.

Diagnosis.—Lobar pneumonia.

Monkey 115 shows that the disease produced by the intratracheal injection of Pneumococcus Type IV is identical with that produced by the other types of pneumococci.

Production of Lobar Pneumonia by Contact Infection.

Epidemiological studies by Stillman[4] have clearly indicated that lobar pneumonia in man caused by Pneumococcus Types I and II is probably due in large part to direct or indirect contact infection with other cases of pneumonia caused by these organisms. It was therefore decided to determine whether lobar pneumonia could be experimentally produced in monkeys by contact infection.

Experiment 15.—May 26, 1919. Monkeys 16, 19, 20, 106, 107, and 108 (*Macacus syrichtus*), all well and active, placed in a cage 4 feet by 4 feet by 3 feet. May 27, 10 a.m. Monkeys 109 and 110 (*Macacus syrichtus*) injected intratracheally with 0.001 and 0.000001 cc. of 18 hour broth culture of Pneumococcus Type I, respectively, and placed in the same cage. May 28. Monkeys 109 and 110 have developed pneumonia. Contacts appear well. May 30. Contacts remain well. Monkey 109 died. June 1. Monkey 19 has developed spontaneous lobar pneumonia, Pneumococcus Type IV; removed from cage.[5] Other contacts remain well. June 3. Monkey 110 died. Contacts remain well. June 5. Monkey 112 (*Macacus syrichtus*) injected intratracheally with 0.01 cc. of an 18 hour broth culture of Pneumococcus Type I, and placed in cage. Contacts remain well. June 6. Monkey 112 has developed pneumonia. 3.45 p.m. Contact Monkey 16 appears sick; breathing rapidly. White blood corpuscles 59,700; blood culture shows Pneumococcus Type I (Text-fig. 15). Other contacts well. June 7. Monkey 16 sick; respirations rapid and labored. June 8 to 10. Monkey 16 running typical course of lobar pneumonia. Other contacts remain well. June 10. Monkey 112 died. June 11. Monkey 16 recovered. June 12. Monkey 16 killed. *Autopsy.*—Lobar pneumonia, right lower lobe, resolving. June 20. Contacts remain well. Monkey 114 (*Macacus syrichtus*) injected intratracheally with 0.000001 cc. of an 18 hour broth culture of Pneumococcus Type I, and placed in cage. Developed pneumonia and died on June 25. June 26. Contacts remain well.

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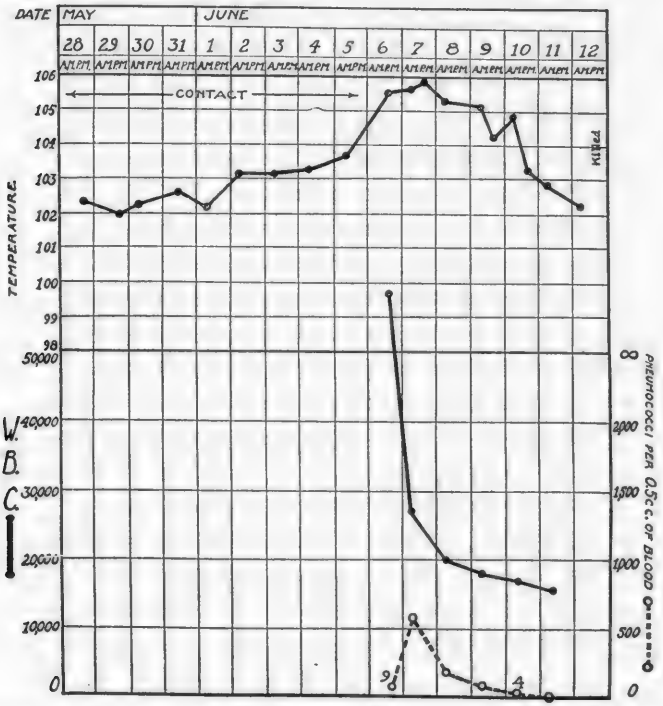
[4] Stillman, E. G., *J. Exp. Med.*, 1916, xxiv, 651; 1917, xxvi, 513.

[5] See Paper III (in press).

The production of lobar pneumonia in Monkey 16 by contact infection eliminated the one minor artificial procedure resorted to in the preceding experiments; namely, the introduction of a needle into the trachea. The fact that only one of the six monkeys exposed contracted Pneumococcus Type I pneumonia indicates clearly that



TEXT-FIG. 15. Monkey 16. Spontaneous Pneumococcus Type I lobar pneumonia following contact with cases of experimental Pneumococcus Type I pneumonia.

other factors besides contact play a part in determining whether or not pneumonia will develop. In contrast with this result the constancy with which lobar pneumonia was produced by the intratracheal injection of even very minute amounts of pneumococcus culture clearly indicates that a virulent pneumococcus once having gained entrance to the respiratory tract below the larynx readily produces pneu-

monia in susceptible individuals. Study of the pathogenesis of experimental lobar pneumonia presented in Paper II indicates that it must penetrate as far as the larger bronchi within the lung before pneumonia will develop. The factors which determine whether or not a pneumococcus will gain access to the lower respiratory tract have not been studied. The following experiment clearly shows that its mere presence in the upper respiratory tract even over considerable periods of time is not sufficient in itself to cause even a highly susceptible animal to develop pneumonia.

Effect of Inoculating Monkeys in the Nose and Throat with Virulent Pneumococcus.

Experiment 16.—June 26, 1919. Monkeys 20, 106, 107, and 108 (*Macacus syrichtus*). All well and active. 10.20 a.m. Nose and throat of each monkey sprayed with 2 to 4 cc. of an 18 hour broth culture of Pneumococcus Type I. Monkeys 20, 106, and 108 were held under observation until July 28, Monkey 107 until July 1. All remained perfectly well throughout this period, showing no evidence of pneumonia or of infection of the upper respiratory tract. From time to time saliva was collected and injected intraperitoneally into white mice to determine whether or not Pneumococcus Type I was still present. The results are shown in Table II.

TABLE II.

Persistence of Pneumococcus in the Mouth of Normal Monkeys.

Monkey No.	Date of inoculation.	Pneumococcus Type I in mouth.			
		June 30.	July 7.	July 18.	July 28.
	1919				
20	June 26	+	+	+	+
106	" 26	+	+	+	+
107	" 26	+			
108	" 26	+	+	−	+

The continued existence of a virulent Pneumococcus Type I in the mouths of monkeys for a period of a month without any of them contracting pneumonia is of interest. In connection with experiments with *Bacillus influenzæ* reported in Paper IX it seems advisable to call attention at this point to the fact that a highly virulent pneumococcus inoculated in large amounts into the nose and mouth of animals highly susceptible to pneumococcus infection had absolutely no effect upon them whatsoever.

TABLE III.

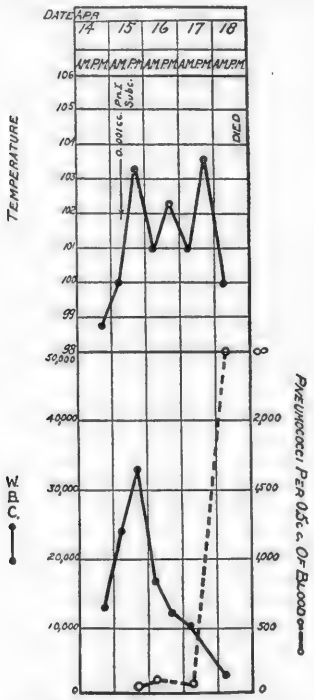
Effect of Intravenous and Subcutaneous Inoculation of Monkeys with Pneumococcus Type I.

Monkey No.	Species.	Weight.	Date of inoculation.	Route of inoculation.	Amount of culture.	Result.	Autopsy.
		gm.	*1919*		*cc.*		
7	*C. capucinus.*	1,430	Mar. 7	Subcutaneous.	0.001	Pneumococcus septicemia. No evidence of pneumonia. X-ray negative. Mar. 14. Recovered.	No autopsy.
46	*M. syrichtus.*	2,550	Apr. 15	"	0.001	Pneumococcus septicemia. Apr. 18. Died.	Pneumococcus septicemia. Lungs normal.
47	" "	2,710	" 15	"	0.001	Temporary febrile reaction and leucocytosis of 24 hrs. duration. Blood sterile.	No autopsy.
48	" "	3,590	" 15	"	0.001	Temporary leucocytosis. No other apparent effect.	" "
8	*C. capucinus.*	1,065	Mar. 7	Intravenous.	0.01	Pneumococcus septicemia. Mar. 8. Died.	Pneumococcus septicemia. Lungs normal.
49	*M. syrichtus.*	4,120	Apr. 15	"	0.001	Pneumococcus septicemia. Apr. 22. Died.	Pneumococcus septicemia. Lungs normal.
104*	" "	1,735	July 8	"	0.001	Temporary pneumococcus septicemia. July 11. Recovered. July 14. Killed.	No lesions.
105*	" "	1,375	" 8	"	0.001	Temporary leucocytosis. No other apparent effect. July 12. Killed.	" "
116	" "	1,935	" 8	"	0.001	Pneumococcus septicemia. No evidence of pneumonia. July 14. Recovered. July 15. Killed.	" "

* Vaccinated with 1 cc. of Pneumococcus Type I saline vaccine (1,000,000,000 pneumococci) on May 22, 29, and June 5, respectively.

Effect of Intravenous and Subcutaneous Inoculation of Monkeys with Virulent Pneumococcus.

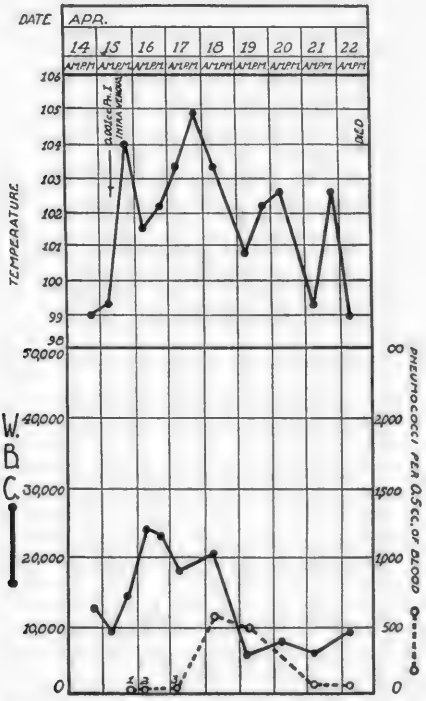
Previous attempts to produce lobar pneumonia in normal animals by intravenous or subcutaneous inoculation of pneumococcus have met with consistent failure.[3] In spite of this fact the theory that the



TEXT-FIG. 16. Monkey 46. Effect of the subcutaneous injection of 0.001 cc. of Pneumococcus Type I broth culture.

disease in man is hematogenous rather than bronchiogenic in origin has from time to time been advanced, although without convincing evidence to support it. It therefore seemed advisable to control the results obtained by intratracheal injection by a series of experiments to determine the effect of intravenous and subcutaneous inoculation of pneumococcus in monkeys. Five monkeys received intravenous in-

jections and four monkeys subcutaneous injections of 0.01 to 0.001 cc. of an 18 hour broth culture of Pneumococcus Type I, the same strain being used as in the preceding experiments, all with entirely negative results in as far as the production of pneumonia was concerned. The results are shown in Table III. Individual variation in susceptibility probably explains the different results obtained.



TEXT-FIG. 17. Monkey 49. Effect of the intravenous injection of 0.001 cc. of Pneumococcus Type I broth culture.

Two illustrative protocols with accompanying clinical charts are presented.

Experiment 17. Monkey 46 (Text-Fig. 16).—Macacus syrichtus, female; weight 2,550 gm. Apr. 14, 1919. Well and active. Apr. 15, 10 a.m. Subcutaneous injection of 0.001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus

Type I. Apr. 16. Quiet but otherwise appears well. Apr. 17. Appears moderately sick; breathing normally; chest clear. Apr. 18. Sick; respirations slow; chest clear; abdomen slightly distended. Apr. 19. Found dead in morning.

Autopsy.—No lesions; lungs normal; pneumococcus septicemia.

Cultures.—Heart's blood, Pneumococcus Type I; lungs, no growth.

Experiment 18. Monkey 49 (Text-Fig. 17).—*Macacus syrichtus*, male; weight 4,120 gm. Apr. 14, 1919. Well and active. Apr. 15, 10.30 a.m. Intravenous injection of 0.001 cc. (in 1 cc.) of 18 hour broth culture of Pneumococcus Type I. Apr. 16. Quiet; respirations normal; chest clear. Apr. 18. Moderately sick; no definite symptoms; chest clear. Apr. 19 to 21. Condition the same. Apr. 22. 1 p.m. Very sick; lying on floor of cage. 3 p.m. Died.

Autopsy.—No lesions; lungs normal; pneumococcus septicemia.

Culture.—Heart's blood, Pneumococcus Type I.

Monkeys 46 and 49 show that pneumococci injected subcutaneously or intravenously exhibit no tendency to localize in the lungs, or, in fact, anywhere in the body, but produce only a pneumococcus septicemia. Symptoms characteristic of lobar pneumonia were entirely lacking in both animals throughout the course of the infection as they have been in other animals so inoculated.

Effect of Intratracheal Injection of Sterile Broth, Killed Pneumococcus Culture, and Living Avirulent Pneumococcus.

A few further experiments to control the results obtained by intratracheal injection of living virulent pneumococci were carried out as shown in Table IV.

The entirely negative results in these experiments show that the direct intratracheal injection of 1 cc. of fluid with or without pneumococcus cells was not in itself in any way responsible for the development of pneumonia in monkeys injected by this method with living virulent pneumococci.

TABLE IV.

Effect of Intratracheal Injection of Sterile Broth, Killed Pneumococcus Culture, and Living Avirulent Pneumococcus.

Monkey No.	Species.	Weight.	Date of injection.	Material injected.	Result.	Autopsy.
		gm.	*1919*			
5	*C. capucinus.*	1,410	Feb. 28	1 cc. of sterile broth.	Slight polymorphonuclear leucocytosis 6 hrs. after injection. No other effect.	No autopsy.
10	" "	827	Mar. 18, 10.45 a.m.	1 cc. of Pneumococcus Type I broth culture previously killed by heating at 55°C. for 45 min.	Slight febrile reaction 6 hrs. after injection. Moderate polymorphonuclear leucocytosis 6 hrs. after injection. No other effect. Mar. 19, 3 p.m. Killed.	Lungs appear normal. Microscopic examination shows no abnormalities.
24	" "	855	Mar. 20, 10.15 a.m.	2 cc. of 18 hr. broth culture of avirulent strain of Pneumococcus Type I.	No febrile reaction. Blood sterile. Moderate polymorphonuclear leucocytosis 6 hrs. after injection. Mar. 21. X-ray of chest negative. Remained well.	No autopsy.

DISCUSSION.

That the disease experimentally produced in monkeys by the intratracheal injection of minute amounts of pneumococcus cultures is clinically identical with lobar pneumonia in man seems quite clear and requires no special comment. Certain features of the disease, however, which have come out during the course of the study require discussion, since they may serve to throw some light on the course of lobar pneumonia in man.

Although it is generally believed that lobar pneumonia is bronchiogenic in origin there has been no certain evidence that such is the case, as the recurring suggestion that it may be hematogenous in origin testifies. The latter conception has apparently depended upon the clinical observation of cases in which the pneumococcus was shown to be present in the blood at the time of onset of clinical symptoms of pneumonia, or in occasional instances before the clinical symptoms of pneumonia had appeared. That the interpretation of this observation as indicative of the hematogenous origin of lobar pneumonia is incorrect is established by the fact that in the majority of instances in pneumonia experimentally produced by the intratracheal injection of pneumococcus, the organisms have appeared in the blood stream within from 6 to 24 hours after injection, frequently before clinical evidence of pneumonia or elevation of temperature had developed. It is presumable that they rapidly gain access to the blood by way of the lymphatics draining from the lungs, a supposition well supported by histological study of the earliest stages of experimental pneumonia presented in the following paper. The constant production of the disease by intratracheal injection and the constant failure to produce it by other methods of inoculation are believed to be conclusive evidence that infection in lobar pneumonia takes place by way of the respiratory passages.

The failure to produce pneumonia by the instillation of large amounts of a virulent pneumococcus culture into the nose and throat of animals as highly susceptible to pneumococcus infection as monkeys, even though the animals so inoculated continued to carry the pneumococcus in the mouth for at least a month, clearly suggests that there are unknown factors which determine whether or not a

virulent pneumococcus in the upper respiratory tract will gain access to the bronchi. No experiments to determine what these factors are were carried out. It seems established by this observation, however, that the pneumococcus is incapable of initiating an infection of the normal upper respiratory tract in monkeys at least, and that it should probably be regarded only as a secondary invader when found associated with such infections in man.

It is generally stated that the degree of leucocytosis in lobar pneumonia in man is inversely proportional to the severity of the pneumonia, but that individual variation is so great that it is of little prognostic value in the given case. Analysis of the leucocytic reaction during the course of the disease in monkeys, however, has shown a rather surprising constancy in the relation between the leucocyte curve and the severity of the infection as measured by the degree of septicemia, the development of complications, and the final outcome. It has been stated that the onset of pneumonia in monkeys is accompanied by an initial leucocytosis. This preliminary leucocytosis, which was polymorphonuclear in character, usually began within 6 hours after injection and reached its apex within 24 to 48 hours. It varied from 35,000 to 90,000 cells per c.mm., the polymorphonuclear neutrophils comprising from 85 to 95 per cent of the leucocytes. It was not possible to establish any apparent relation between the height of this leucocytosis and the subsequent course of the disease. It was well developed in many cases before clinical evidence of pneumonia or elevation of temperature had occurred, in this respect being similar to the frequent early invasion of the blood by pneumococci. There seems little doubt that it occurred as an immediate response on the part of the animal to invasion of the pulmonary tissue by the pneumococcus and represents an effort on the part of the host to combat the infection at the point of entrance. This was shown to be the case in a monkey killed 3 hours after intratracheal injection (Monkey 72, Paper II), the leucocytes having increased from 15,200 per c.mm. to 25,700 per c.mm. in that time. Histological sections of the right lower lobe showed extensive infiltration of polymorphonuclear leucocytes in the peribronchial tissue near the hilum, many leucocytes passing through the mucosa of the bronchial wall, and many already extruded into the lumen of the bronchus. A

similar though more extensive infiltration of leucocytes was observed in several monkeys dying within 48 hours after inoculation. In some of these considerable phagocytosis of pneumococci had already taken place.

Following the preliminary rise in leucocytes there has been a constant fall in the curve. The rapidity and extent of this fall has seemed to bear a direct relation to the severity of the disease and the degree of pneumococcus invasion of the blood. This is clearly illustrated by contrasting the leucocyte curve of Monkey 115 (Text-fig. 1) with that of Monkey 110 (Text-fig. 5). Study of stained blood films has shown that during this fall mature polymorphonuclear leucocytes are increasingly replaced by young forms with two or three nuclear lobules, until in the very severe cases with overwhelming septicemia and marked leucopenia mature leucocytes have practically disappeared from the blood. In the latter group of cases occasional nucleated red blood corpuscles, myelocytes, and polychromatophilic cells simultaneously appear. This phenomenon points to an initial intense stimulation of the bone marrow followed by a progressive exhaustion as the severity of the infection increases. In harmony with this supposition is the observation that the bone marrow of monkeys dying early with an overwhelming septicemia has frequently been almost entirely devoid of polymorphonuclear leucocytes, and myelocytes have been few in number. It would appear that the leucocytes available at the onset of the disease are transported bodily, so to speak, from the bone marrow to the lungs and that the animal is no longer able to respond with further production of leucocytes in the presence of an overwhelming infection. Whether the progressive exhaustion of bone marrow activity is due to the increasing severity of the infection, or the increasing severity of the infection is due to an inherent weakness of the leucocytic defense in the individual animal is uncertain.

In contrast with the foregoing picture is the one presented by less severe cases which progress favorably to recovery. The leucocyte curve instead of continuing progressively downward turns upward again and progressively rises during the latter half of the disease until crisis occurs, when it again falls to normal. Simultaneously with this increase in leucocytes, the degree of septicemia diminishes until the

blood becomes sterile, usually several days before crisis. This phenomenon has been surprisingly constant and has occurred in nearly all the cases that have recovered. It has been pointed out that a few animals exhibiting this secondary rise in the leucocyte curve have shown a continuing septicemia which terminated in death. In this small group of cases autopsy has shown a complicating pericarditis or empyema in most instances. In both these groups the bone marrow has frequently shown greatly increased numbers of myelocytes.

The mechanism of recovery from lobar pneumonia has been the subject of much investigation. Neufeld and Haendel, Cole and his coworkers, Blake, and others[6] have attributed it in part to the development of humoral antibodies and have demonstrated the appearance of these antibodies in the circulating blood at or about the time of crisis. More recently Lord[7] has suggested that another factor may be of considerable importance in bringing about crisis and resolution of the pneumonic process, attributing recovery to local biochemical changes in the course of which the acid death-point of the pneumococcus is reached. Although no study of the mechanism of crisis has been made in the course of the experiments reported, certain isolated observations seem of interest in this connection. In one instance crisis with recovery occurred with pneumococcus septicemia persisting for 48 hours after crisis. In several instances a critical fall in temperature has occurred about the 7th to 9th day with subsequent return of fever and death several days later. Autopsy has shown a resolving pneumonia, death apparently being due to a persisting pneumococcus septicemia usually associated with some complication. In another small group of cases recovery from the general pneumococcus infection has occurred as evidenced by the disappearance of pneumococci from the blood. Crisis, however, did not occur and the monkeys finally died showing an unresolved pneu-

[6] Neufeld, F., and Haendel, *Arb. k. Gsndhtsamte.*, 1910, xxxiv, 166. Dochez, A. R., *J. Exp. Med.*, 1912, xvi, 665. Avery, O. T., Chickering, H. T., Cole, R., and Dochez, A. R., Acute lobar pneumonia. Prevention and serum treatment, Monograph of The Rockefeller Institute for Medical Research, No. 7, New York, 1917. Blake, F. G., *Arch. Int. Med.*, 1918, xxi, 779.

[7] Lord, F. T., *J. Exp. Med.*, 1919, xxx, 379.

monia with pneumococci still present in the lung. These observations, though far from proving the fact, are not out of harmony with the theory advanced by Lord with respect to the mechanism of crisis and suggest that other important factors besides the development of humoral immunity are necessary to bring about recovery from pneumonia. With these points in mind it does not seem unreasonable to look upon lobar pneumonia as comprised of two distinct though intimately related processes; one, always present, being the local pulmonary lesion, the other, present in a variable number of cases, being a general infection of the body as manifested by the frequent pneumococcus septicemia. Though ultimate recovery must primarily depend upon the ability of the individual to prevent or terminate the general infection when once established, presumably through the existence or development of a humoral immunity, it does not follow that recovery from the local process with resolution of the pneumonic consolidation need be either coincident with recovery from the general infection or dependent upon the same mechanism. In fact, it would seem well established by numerous clinical observations that recovery from the general pneumococcus infection when it exists usually precedes, by several days at least, recovery from the disease at the time of crisis. On the other hand, certain of the observations cited above would seem to indicate that recovery from the local process as shown by a rapidly resolving pneumonia may occasionally occur prior to recovery from the general infection or even when death from the general infection subsequently takes place. In view of the above considerations it would seem not improbable that at least a dual mechanism may be concerned in bringing about final recovery from lobar pneumonia. Expression of any certain opinion on the subject, however, must depend on much further work.

SUMMARY.

1. Lobar pneumonia has been consistently produced in normal monkeys by the intratracheal injection of minute amounts of pneumococcus culture.

2. The disease produced has been shown to be clinically identical with lobar pneumonia in man.

3. Lobar pneumonia has been produced in the monkey in one instance by experimental contact infection.

4. Normal monkeys inoculated in the nose and throat with large amounts of pneumococcus culture have failed to develop lobar pneumonia though carrying the organism in their mouths for at least a month. They have likewise failed to show any evidence of upper respiratory tract infection.

5. Monkeys inoculated subcutaneously or intravenously with pneumococcus culture have in no instance developed pneumonia, but have either died of pneumococcus septicemia or recovered without localization of the infection in the lungs.

CONCLUSIONS.

1. The pneumococcus is the specific cause of lobar pneumonia.

2. The pneumococcus is unable to initiate an infection of the normal mucous membranes of the upper respiratory tract or to produce pneumonia following intravenous injection, but must gain access to the lower respiratory tract by way of the trachea in order to cause pneumonia.

3. Lobar pneumonia is, therefore, bronchiogenic in origin.

4. Invasion of the blood stream by the pneumococcus in lobar pneumonia is secondary to infection of the lungs.

5. The character of the leucocyte reaction during the course of lobar pneumonia bears a fairly definite relation to the course of the disease.

EXPLANATION OF PLATE 21.

FIG. 1. Monkey 75. Experimental lobar pneumonia; Pneumococcus Type I. X-ray of the chest showing consolidation of the left lower lobe.

FIG. 2. Monkey 3. Experimental lobar pneumonia; Pneumococcus Type I. X-ray of the chest showing consolidation of the left upper lobe.

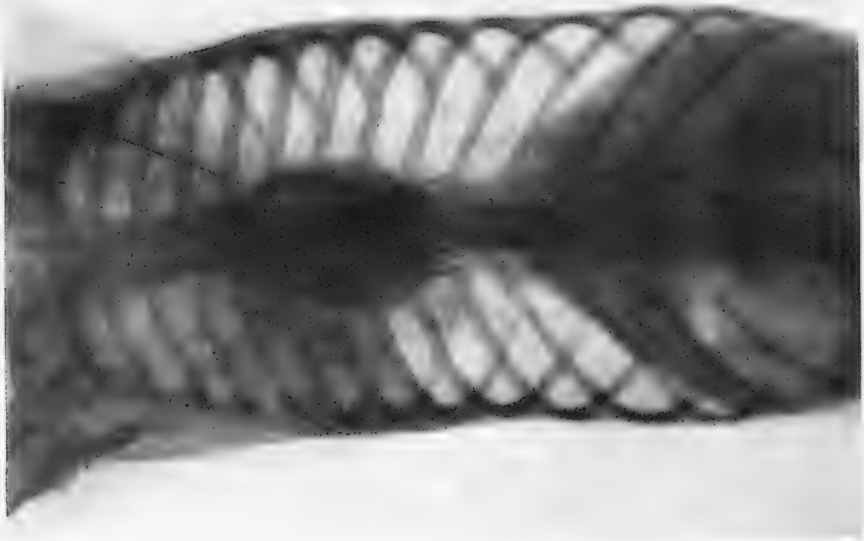


FIG. 2.

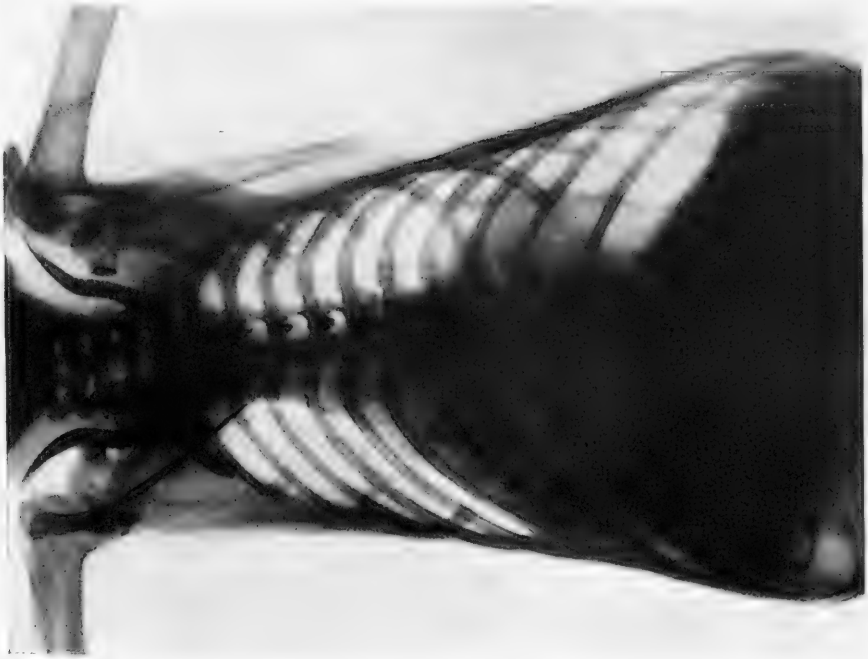


FIG. 1.

(Blake and Cecil: Experimental pneumonia. I.)

UNIV. OF MICH.

STUDIES ON EXPERIMENTAL PNEUMONIA.

II. Pathology and Pathogenesis of Pneumococcus Lobar Pneumonia in Monkeys.

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(*From the Bacteriological Laboratories of the Army Medical School, Washington.*)

Plates 22 to 44.

(Received for publication, January 23, 1920.)

In a preceding paper[1] it has been shown that pneumonia may be produced readily in normal monkeys by the intratracheal injection of minute amounts of pneumococcus culture and that the disease so produced runs a clinical course identical with that of lobar pneumonia in man. In this paper it is proposed, first, to describe the pathology of pneumococcus pneumonia experimentally produced in monkeys, in order to show that the disease is identical with lobar pneumonia in man, and, second, to present observations concerning the pathogenesis of lobar pneumonia based upon study of the pathology of lobar pneumonia in monkeys.

Pathology of Pneumococcus Pneumonia in Monkeys.

Autopsies were performed in 40 cases of pneumonia experimentally produced in monkeys by the intratracheal injection of pneumococcus. Of these, twenty-seven died during the active stage of the disease or later from complications, and thirteen were killed, one on the 9th day of the disease and twelve at varying intervals after recovery by crisis. The cases are summarized in Table I, the fatal cases, including Monkey 26, being arranged according to the duration of the disease, the recovered cases according to the time elapsing between recovery and the killing of the animal for pathological examination. The day of intratracheal injection is considered the 1st day of the disease, since onset of pneumonia had usually occurred within 24 hours after inoculation.

[1] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

Macroscopic Pathology.

Macroscopically the lungs at autopsy presented the characteristic picture of lobar pneumonia (Figs. 1 to 3), showing the stages of engorgement, red hepatization, gray hepatization, and resolution. The stage of pneumonia found at autopsy depended primarily upon the duration of the disease at the time of death. This was modified to a certain extent, however, by the amount of pneumococcus culture injected, monkeys receiving a comparatively large amount showing a more advanced involvement early in the disease than those receiving the smaller amounts.

It will be seen from Table I that the lungs of monkeys dying within 5 days after intratracheal injection presented the macroscopic picture of engorgement or red hepatization, and that gray hepatization appeared only subsequently to this. In reality, of course, no such sharp distinction could be made between the different stages of the disease, since, as is well recognized,[2] lobar pneumonia is a progressive process and one stage merges gradually into the next. This was readily observed on examination of the cut surfaces of the lungs and is well illustrated in Fig. 2 in which the right lower lobe shows both red and gray hepatization.

Of the monkeys killed at varying intervals after recovery, five showed an actively resolving pneumonia, two resolution combined with partial organization, and one extensive organization. Organizing pneumonia was also found in two fatal cases (Monkeys 14 and 42) which died at a considerable interval after apparent recovery from the active stage of the disease. The involved lobes in these cases presented a characteristic translucent, grayish appearance, being of a doughy consistency when resolution was occurring, firm and rubbery when organization was taking place.

In three cases of mild pneumonia (Monkeys 82, 96, and 107), in two of which the disease presented the clinical picture of an abortive attack, the lungs showed an interstitial involvement with little or no exudate in the alveoli. In the remaining case (Monkey 75) the pathological picture was complicated by an extensive pulmonary tuberculosis.

[2] Delafield, F., and Prudden, T. M., A text-book of pathology, New York, 10th edition, 1914, 606.

Tabulation of the lobes involved (Table II) shows that in both the fatal and recovered cases the right and left sides were about equally affected and that the lower lobes were about twice as frequently involved as the middle or upper lobes. The extent of the involvement, however, was in striking contrast in the two groups. Of the twenty-eight fatal cases (including Monkey 26) seventeen showed bilateral pneumonia, while of the twelve recovered cases only three showed involvement on both sides. Eighteen of the fatal cases showed pneumonia of three or more lobes, while none of the recovered cases showed involvement of more than two lobes, and in more than half the pneumonia was confined to one lobe.

The distribution and extent of the pneumonic lesions within the lobe in the different stages of the disease has also been studied. In the earliest stages of engorgement before hepatization had begun the most striking feature was a thickening of the vascular adventitia and walls of the bronchi near the root of the lobe (Figs. 4 to 6). This process spread rapidly throughout the lobe, following the ramifications of the vascular and bronchial trees and appeared always to precede exudation into the alveoli. The interlobular septa were similarly, though less conspicuously, involved. Microscopic examination showed that it was due to edema and leucocytic infiltration of the interstitial tissue. It was accompanied by intense capillary engorgement.

By the time this process had extended throughout the lobe hepatization usually had begun. In monkeys that died early in the stage of red hepatization the consolidation was found invariably to occupy the portions of the lobe proximal to the hilum (Figs. 7 and 8). Surrounding the consolidated areas was a zone of edematous alveoli. In the distal portions of the lobe engorgement was found, the alveoli being still air-containing.

With the further progress of the disease the distal portions of the lobe became progressively consolidated until complete lobar hepatization resulted. By the time red hepatization of the distal portions of the lobe had occurred the portions proximal to the hilum and adjacent to the larger bronchi and vessels were found to have assumed the appearance of gray hepatization (Fig. 2). Finally, in monkeys which died after the 6th day of the disease complete lobar

TABLE I.

Autopsy Findings in Experimental Pneumococcus Lobar Pneumonia in Monkeys.

Monkey No.	Date of inoculation.	Type of pneumococcus.	Amount injected.	Day of disease on which death occurred.	Bacteriology.			Lobes involved and stage of disease.						Complications.
					Heart's blood.	Lungs.	Miscellaneous.	L. U.*	L. M.	L. L.	R. U.	R. M.	R. L.	
	1919		*cc.*											
2	Feb. 24	I	0.5	1st	Pn. I	R. L., Pn. I.	Br., Pn. I.	R. H.		R. H.	R. H.		R.H.	
5	Mar. 5	I	0.01	1st	"	R. U., "	" "			E.	E.		"	
1	Feb. 17	I	1.0	2nd	"	R. L., "							"	
3	" 24	I	0.1	2nd	"	L. U., "		R. H.		E.				
29	Apr. 2	I	1.0	2nd	"		Br., Pn. I.	E.	E.	R. H.				
109	May 27	I	0.001	3rd	"	R. M., Pn. I.	" "	"	"		E.	E.		
6	Mar. 5	I	0.001	4th	"	R. L., "	Tr., "				"		R. H.	
17	" 26	I	1.0	4th	"	" "	Pericard., Pn. I.	E.	E.	E.	"	E.	E.	Ac. fib. pericard.
88	May 20	I	0.000001	4th	"	L. L., "	Br., Pn. I.	"	"	"			"	
98	" 20	I	0.000001	4th	"	" "	" "	"		"	E.		"	
23	Mar. 18	I	1.0	5th	"	" "				R. H.			"	
87	May 6	I	0.1	5th	"	" "	Br., Pn. I.	R. H.	R. H.	"			E.(C.).	
101	June 20	I	0.000001	5th	"	" "	" "	E.	E.	E.			E.	
						R. L., "								
114	" 20	I	0.000001	5th	"	L. L., "	" "			"			"	
					S. H.	S. H.	S. H.							
85	May 6	I	0.001	6th	Pn. I	L. L., Pn. I.	Perit., Pn. I.		R. H.	"				Ac. fib. perit.
112	June 5	I	0.01	6th	"	R. L., "				"		R. H.	R. H.	
												G. H.	G. H.	
110	May 27	I	0.000001	7th	"	" "	Br., Pn. I.				R. H.	"	"	Ac. fib.-pur. pericard.
							Pericard., Pn. I.							
83	Apr. 30	I	0.001	8th	"	" "	Pl., Pn. I.				E.	"	R. H.	Serofib.
											R. H.		G. H.	pleurisy.

26	Mar. 21	I	1.0	9th (K.)	N. G.	L. L., N. G. R. L., "	Br., Pn. I.			G. H.			G. H.	
31	Apr. 2	I	0.01	10th	Pn. I	L. L., "	Emp., " Br., "	G. H.	G. H.	"				Emp.; mediast.
100	June 20	I	0.000001	10th	"	" "		R. H.	"	"			E.	
67	May 6	I	0.001	11th	"	R. L., Pr. I.	Br., Pn. I.						G. H.	
30	Apr. 2	I	0.1	12th	N. G.	" N. G. L. L., "		G. H.	G. H.	G. H. Res.	G. H.	G. H.	" Res.	Hyp. and dil. of heart.
80	May 13	I	0.000001	12th	Pn. I	" Pn. I.	Pericard., Pn. I. Br., Pn. I.		"	G. H.			G. H. (C.).	Ac. fib.-pur. pericard.
93	" 15	I	0.001	13th	"	R. L., "	" "				G. H.	G. H.	G. H.	Hyp. and dil. of heart.
86	" 6	I	0.000001	14th	N. G.	L. L., "	" "			G. H.			"	
14	Mar. 26	I	1.0	17th	Pn. I	R. L., N. G.	" N. G.						Org.	
42	Apr. 10	I	0.001	37th	"	L. L., Pn. I.	" Pn. I.		Org.	Org.	Res.	Res.	Res. Org.	
25	" 4	II	0.1	C. 7th K. 7th	N. G.	R. U., N. G.	" N. G.				"			
95	May 13	I	0.00001	C. 11th R. 15th C. 21st K. 23rd	"	L. L., "				Res. Org.				

* L. U., L. M., L. L., etc., indicate lobes of the lung. The cardiac lobe is included as part of the right lower lobe. (C.) indicates pneumonia of the cardiac lobe without involvement of the right lower lobe. Pn. I indicates Pneumococcus Type I; S. H., *Streptococcus hæmolyticus*. Br. indicates bronchus; Tr., trachea; Pl., pleura; N. G., no growth in cultures. E., R. H., G. H., Res., and Org. indicate engorgement, red hepatization, gray hepatization, resolution, and organization, respectively. The stage of pneumonia recorded shows the predominant type of lesion in the lobe as judged by macroscopic examination. K. indicates killed; C., crisis; R., relapse; L., lysis. The other abbreviations are self-explanatory.

TABLE I—*Concluded.*

Monkey No.	Date of inoculation.	Type of pneumococcus.	Amount injected.	Day of disease on which death occurred.	Bacteriology.			Lobes involved and stage of disease.						Complications.
					Heart's blood.	Lungs.	Miscellaneous.	L. U.*	L. M.	L. L.	R. U.	R. M.	R. L.	
	1919		*cc.*											
82	Apr. 30	III	0.1	C. 7th† K. 8th	N. G.		Tr., N. G.				Res.			
107	July 1	III	1.0	L. 4th‡ K. 5th	"	R. L., N. G.	Br., "						Res.	
96	" 1	III	1.0	L. 3rd‡ K. 5th	"	L. U., "	" "	Res.			Res.			
64	May 6	I	0.000001	L. 27th K. 30th	"	L. L., Pn. I.	" Pn. I.			Org.				
65	" 6	I	0.1	C. 8th K. 12th	"	R. L., N. G.							Res.	
89	" 20	I	0.001	C. 12th K. 16th	"	" "							"	
7	" 27	IV	0.1	C. 8th K. 13th	"	" "	Br., N. G.			Res.			"	
78	" 15	I	0.001	C. 9th K. 15th	"	" "	" "		Res.				" Org.	
81	" 13	I	0.0001	C. 10th K. 17th	"	" "	" "					Res.	Res.	
75	June 24	II	0.1	C. 8th K. 22nd	"				Res.	Res.				Pulmon. tuberc.

† Interstitial and lobular pneumonia.
‡ Interstitial pneumonia.

consolidation in the stage of gray hepatization was encountered (Figs. 3 and 9).

Pleurisy.—Acute fibrinous pleuritis was almost universally found at autopsy. The fibrinous exudate varied considerably in thickness, depending upon the duration of the disease, and was spread in an easily detachable layer over the surfaces of the involved lobes. In seven cases that died very early there was no evident exudate on the

TABLE II.

Extent and Distribution of Pneumonia in Fatal and Recovered Cases of Experimental Lobar Pneumonia in Monkeys.

	Fatal cases (including Monkey 26).		Recovered cases.	
No. of cases	28		12	
Left lung involved	21		6	
Right " "	24		9	
Bilateral involvement	17		3	
One lobe involved	3		7	
Two lobes "	7		5	
Three or more lobes involved	18		0	
Left upper lobe involved	12	23	1	4
Right " " "	11		3	
Left middle " "	12	20	2	3
Right " " "	8		1	
Left lower " "	20	43	4	10
Right " and cardiac lobes involved	23		6	

pleura, but microscopic examination showed an early pleurisy. In cases killed after recovery the exudate was undergoing organization.

Complications.—Pneumococcus complications occurred in six cases. Monkey 31 had empyema, Monkey 83 serofibrinous pleurisy, Monkey 17 acute fibrinous pericarditis, Monkeys 80 and 110 acute fibrinopurulent pericarditis, and Monkey 85 localized subdiaphragmatic fibrinous peritonitis. Pneumococci were isolated in pure culture from these complications, which were similar in all respects to like complications of lobar pneumonia in man.

Other Lesions.—Of extrapulmonary lesions the most striking were in the lymph nodes about the hilum and in the bone marrow. The

lymph nodes were consistently enlarged and succulent. Microscopic examination showed that this enlargement was due to distention of the lymph sinuses with numerous large mononuclear cells and lymphocytes, and with moderate numbers of polymorphonuclear leucocytes (Fig. 33). In many instances the large mononuclear cells showed active phagocytosis of polymorphonuclear leucocytes. This process would appear to represent an accumulation of cells brought to the lymph nodes about the hilum by the lymphatic drainage of the lung. Sections of bone marrow taken from the femur showed the widest variation in hematopoietic activity. In some instances the picture was one of almost complete exhaustion, the marrow being entirely devoid of polymorphonuclear leucocytes and largely so of myelocytes (Fig. 34). In others there was marked hyperplasia with greatly increased numbers of leucocytes and myelocytes in the marrow (Fig. 35). All grades of variation between these two extremes were encountered. The picture found depended upon the course of the disease and the degree of infection at the time of death. In monkeys which died during the active stage of the disease with overwhelming pneumococcus septicemia and terminal leucopenia myeloid exhaustion was found. In monkeys which died later without an early overwhelming septicemia but usually with some complication, the picture was one of myeloid hyperplasia. This finding was in keeping with the secondary rise in the leucocyte count exhibited in these cases.

In two animals (Monkeys 30 and 93) marked dilatation and hypertrophy of the heart were found. Both ran a prolonged severe course. Monkey 30 was of particular interest because death occurred suddenly 4 days after apparent recovery by crisis. Autopsy showed nearly complete consolidation of all lobes, cultures from the lungs and heart's blood being sterile. In several other cases running a prolonged severe course, moderate dilatation of the right heart was found, but hardly sufficient to be of certain significance.

Lesions of the abdominal viscera except for cloudy swelling of the liver and kidneys and congestion of the spleen were not encountered.

Microscopic Pathology.

Microscopically, the pneumonia produced in monkeys by the intratracheal injection of pneumococcus likewise showed the characteristic picture of lobar pneumonia in the stages of engorgement, red hepatization, gray hepatization, and resolution or organization. The progressive nature of the process was well shown in monkeys which died at a moderately advanced stage of the disease, in which the microscopic picture of gray hepatization was found in the alveolar tissue proximal to the hilum, of red hepatization in the surrounding zone of tissue, and of engorgement and edema in the more distal portions of the lobe, the peripheral alveoli at the margins most distant from the hilum often being still air-containing.

Engorgement.—As stated above the most striking feature of the stage of engorgement was the perivascular edema and leucocytic infiltration. The walls of the bronchi and the interlobular septa were similarly involved, and extension of the process to the pleura had occurred where the distance from the root of the lobe to the pleura was not great. This interstitial process was particularly conspicuous about the vessels and involved both arteries and veins (Figs. 11, 13, 14, and 15). The peribronchial process (Fig. 12), always less conspicuous than the perivascular one, was usually most extensive in the tissue lying between the bronchus and its accompanying pulmonary artery, this relation being maintained throughout the course of the bronchi to the terminal bronchioles (Fig. 15). The leucocytic infiltration was composed largely of polymorphonuclear leucocytes. Large mononuclear cells and lymphocytes, however, were always present. In a few cases a considerable degree of hemorrhage in the perivascular and peribronchial tissue was found. Fibrin was also present. Necrosis of the tissue was not encountered. The perivascular, peribronchial, and septal lymphatics were distended and often contained large numbers of leucocytes (Figs. 16, 17, and 23).

The epithelium of the bronchi and bronchioles appeared comparatively intact. The bronchi and bronchioles contained little or no exudate (Fig. 15). The vessels and the capillaries of the alveolar walls were greatly engorged (Fig. 18). In the parenchymal tissue

proximal to the hilum the walls of the alveolar ducts and of the adjacent alveoli were often swollen and infiltrated with polymorphonuclear leucocytes, variable numbers of large mononuclear cells, and occasional lymphocytes (Figs. 22 and 23). Some of the alveoli in these areas contained a few large mononuclear cells which appeared to be desquamated alveolar epithelium; otherwise they contained no exudate.

Red Hepatization.—With the further progress of the disease to the stage of red hepatization the more familiar histological pictures of lobar pneumonia appeared. Exudation into the alveoli occurred first in the parenchymal tissue proximal to the hilum, and subsequently progressively involved the more distal portions. In the earliest stages of hepatization the alveoli contained coagulated serum, and the alveolar walls frequently were infiltrated with polymorphonuclear leucocytes and large mononuclear cells. In areas where hepatization was somewhat more advanced the alveoli contained variable numbers of polymorphonuclear leucocytes, large mononuclear cells, and red blood corpuscles embedded in a network of fibrin (Figs. 25, 27, and 28). Frequently the large mononuclear cells contained phagocytosed leucocytes or red blood corpuscles. The character of the mononuclear cells in the exudate and the fact that similar cells were present in considerable numbers in the bronchial walls, perivascular tissue, and alveolar walls during the stage of engorgement, indicate that they were probably, at least in large part, endothelial leucocytes. Some, however, appeared to be desquamated, alveolar epithelial cells.

The number of red blood corpuscles in the exudate varied greatly in different cases and in different areas in the same case. The almost complete absence of red blood corpuscles in the alveolar exudate in some cases in the stage of red hepatization makes it probable that the color of the lung in this stage is largely due to the greatly congested alveolar capillaries.

The interstitial lesions so prominent during the stage of engorgement, though still present in the stage of red hepatization, were much less conspicuous where hepatization had occurred. At the margins of the advancing pneumonic consolidation, however, the primarily interstitial character of the lesion was clearly evident, the peri-

bronchial and perivascular tissue and the alveolar walls being edematous and infiltrated with leucocytes to a variable extent. The lumina of terminal bronchioles and alveolar ducts in many places were still free from exudate, in others where red hepatization was well advanced they contained an exudate similar to that found in the alveoli.

Gray Hepatization.—With still further advance of the process the number of polymorphonuclear leucocytes in the alveolar exudate increased, the fibrin threads often became granular and degenerated, the congestion of the alveolar capillaries disappeared, and the picture passed to that of typical gray hepatization.

The transition from red to gray hepatization occurred first in the portions of the lobe where red hepatization first developed; that is, in the parenchymal tissue proximal to the hilum. Subsequently the more distal portions of the lobe assumed the appearance of gray hepatization. In this stage the alveoli were filled with masses of polymorphonuclear leucocytes. Small numbers of large mononuclears, lymphocytes, and red blood corpuscles were also present. The alveolar walls were thin and appeared well preserved, their capillaries being no longer engorged (Fig. 29). The alveolar ducts and terminal bronchioles were filled with an exudate of leucocytes, red blood corpuscles, and fibrin, and the bronchi contained a variable amount of polymorphonuclear leucocytes and granular debris. The epithelium of the bronchioles and bronchi appeared comparatively intact. Necrosis of the alveolar structure or of the bronchial walls was not found. Edema and leucocytic infiltration of the peribronchial and perivascular tissue, though still present to some extent, were much less conspicuous than in the earlier stages of the disease. The pleura was covered with a dense exudate of fibrin in which polymorphonuclear leucocytes were embedded.

Resolution.—The picture of gray hepatization passed progressively to that of resolution without sharp demarcation between them. In monkeys which died late in the disease or were killed shortly after recovery the alveolar exudate was undergoing rapid disintegration and consisted of leucocytic, nuclear elements and granular debris, occasional lymphocytes, and red blood corpuscles. The alveolar epithelium showed active proliferation and desquamation. As the

process of resolution progressed, desquamated epithelial cells were frequently the predominating cells in the alveoli, the preceding exudate having largely disappeared (Fig. 30). The bronchioles and bronchi were filled with polymorphonuclear leucocytes, mononuclear cells, and desquamated alveolar epithelium in varying proportions. The peribronchial and perivascular tissue was again conspicuous, being infiltrated with large numbers of lymphocytes and plasma cells, and to some extent with polymorphonuclear leucocytes. In still later stages of resolution the alveoli were largely devoid of exudate, containing only here and there a few desquamated epithelial cells. The bronchi and bronchioles no longer showed exudate in their lumina. The perivascular and peribronchial tissue was still conspicuously infiltrated with plasma cells and lymphocytes.

Organization.—Coincident with resolution a variable degree of organization was always found. This was consistently present in the perivascular tissue, though varying greatly in extent, even when complete resolution of the alveolar exudate had taken place. New connective tissue formation about the bronchi also occurred to a considerable extent in cases which showed a widespread organizing pneumonia. Organization of the alveolar exudate, when it occurred, was usually of patchy distribution, alveoli filled with plugs of young connective tissue lying side by side with those in which resolution was rapidly progressing (Fig. 31). The masses of organizing alveolar exudate were covered by a thin layer of epithelium apparently derived from the alveolar walls. In three cases a very extensive organizing pneumonia was found, the normal structure of the lung parenchyma being replaced by young connective tissue in which the distorted remains of alveoli might here and there be found (Fig. 32). The pleural exudate was undergoing organization.

In the following paragraphs autopsy protocols illustrating the different stages of the disease are presented.

PROTOCOLS.

Protocol 1.—Monkey 98. *Macacus syrichtus*, male; weight 4,100 gm. May 20, 1919. Intratracheal injection of 0.000001 cc. of Pneumococcus Type I. Developed pneumonia with severe pneumococcus septicemia and died on May 23.

Anatomical Diagnosis.—Lobar pneumonia, left upper, left lower, right upper, and right lower lobes; stage of engorgement.

Pleural cavities.—Contain no fluid. Pleural surfaces are for the most part glistening, but in scattered patches over the upper and lower lobes appear dull. There is no evident deposit of fibrin. *Right lung.*—Somewhat collapsed. The upper lobe feels soggy in its posterior portion, but shows no consolidation. The posterior and inferior surfaces present blotchy dark red areas extending outward from the hilum toward the periphery. The cut surface appears intensely congested and yields a moderate amount of blood-tinged frothy fluid. The walls of the larger vessels and bronchi appear edematous. The alveoli are air-containing. The lower lobe presents a similar picture, the upper, posterior, and external surfaces showing deep red, blotchy areas extending from the hilum toward the periphery. The cut surface shows marked congestion and yields a moderate amount of fluid. The walls of the larger vessels and bronchi appear edematous. The alveoli are air-containing. The bronchi contain a small amount of blood-tinged mucus. The middle lobe is pale pink and crepitant throughout. *Left lung.*—The upper and lower lobes show an involvement similar in character to that found on the right. There is no consolidation. The left middle lobe appears normal. *Bronchial lymph nodes.*—Enlarged and soft; pinkish gray in color. *Other organs.*—Pericardium and heart show no changes. Liver and kidneys show cloudy swelling.

Cultures.—Heart's blood, bronchi, and left lower lobe, Pneumococcus Type I.

Macroscopic Examination.—Examination of a large histological section from the right lower lobe (Fig. 4) shows marked thickening of the perivascular tissue. This is particularly conspicuous about the larger arteries and veins near the hilum, but is also present about the smaller vessels in the more peripheral portions of the lobe. The walls of the large and medium sized bronchi are similarly thickened, though much less conspicuously so. The bronchi and bronchioles contain no exudate. Many of the interlobular septa appear moderately thickened. The alveoli are air-containing.

Microscopic Examination.—Right upper lobe.—The pleura in sections near the root of the lobe shows capillary engorgement, moderate edema, and infiltration with polymorphonuclear leucocytes, large mononuclear cells, and occasional lymphocytes. This process is especially prominent at the junction of interlobular septa with the pleura. The pleural surface appears intact and there is no fibrinous exudate. The adventitia of the veins and arteries is greatly distended with edema and intensely infiltrated with polymorphonuclear leucocytes and varying numbers of large mononuclear cells and lymphocytes. This process is most conspicuous about the larger vessels, but is also present about the smaller vessels throughout the lobe (Figs. 13 and 14). The perivascular lymphatics are greatly distended and filled with leucocytes (Fig. 17) which in places are embedded in fibrin. The interlobular septa are edematous and infiltrated with abundant polymorphonuclear leucocytes, moderate numbers of large mononuclear cells, and occasional lymphocytes (Fig. 16). Their lymphatics are distended and filled with leucocytes. The walls of the larger bronchi near the root of the lobe

are edematous and infiltrated with leucocytes (Fig. 12). The walls of the smaller bronchi and bronchioles show little or no edema and only moderate leucocytic infiltration (Fig. 15). The epithelium of the bronchi and bronchioles appears intact. The bronchioles contain no exudate. In some of the larger bronchi a few polymorphonuclear leucocytes are seen penetrating between the epithelial cells. The alveoli contain no exudate, except here and there a few large mononuclear cells. The alveolar capillaries are intensely engorged (Fig. 18). Where the engorgement is most extreme the alveoli often appear atelectatic. The alveolar walls show no leucocytic infiltration, except in a few places adjacent to the vessels or bronchi. Moderate numbers of pneumococci are seen in the perivascular and peribronchial tissue, some free, others within polymorphonuclear leucocytes. Occasional pairs are seen in the interlobular septa. in the pleura, and in alveolar walls adjacent to the larger vessels. A few are entangled in the cilia of the epithelium of the larger bronchi. None are seen in the smaller bronchi, bronchioles, or alveolar spaces. *Right lower lobe.*—Presents a similar picture. *Left lung.*—Sections closely resemble those from the right lung. *Bronchial lymph nodes.*—The marginal lymph sinuses are filled with large mononuclear phagocytes, many of which contain polymorphonuclear leucocytes. Many lymphocytes and a few polymorphonuclear leucocytes are also present. No pneumococci are seen.

Protocol 2.—Monkey 23. *Macacus syrichtus*, male; weight 3,555 gm. Mar. 18, 1919. Intratracheal injection of 1 cc. of Pneumococcus Type I. Developed pneumonia with pneumococcus septicemia and terminal leucopenia. Mar. 23, 1 a.m. Died.

Anatomical Diagnosis.—Lobar pneumonia, left lower lobe, red hepatization; right lower lobe, engorgement; acute fibrinous pleuritis, left.

Left pleural cavity.—Contains no fluid. There is a thin layer of fresh fibrin over the posterior and external surfaces of the left lower lobe. *Right pleural cavity.*—Contains no fluid. Pleura is smooth and glistening. *Left lung.*—The posterior two-thirds of the left lower lobe from the hilum nearly to the base is firmly consolidated, the surface dark red in color. The anterior portion is crepitant and air-containing. On section the consolidated portion is dark red and finely granular, the alveoli being filled with plugs of exudate. The base and anterior portions are congested, and yield a considerable amount of bloody, frothy fluid. The perivascular tissue appears edematous. The interlobular septa are not prominent. The bronchi contain a moderate amount of bloody mucoid material. The upper and middle lobes are pale pink and crepitant. *Right lung.*—The upper and posterior surfaces of the lower lobe are mottled with deep red patches. The cut surface shows congestion and moderate edema at the root of the lobe. There is no consolidation. The upper and middle lobes appear normal. *Bronchial lymph nodes.*—Enlarged and soft; pinkish gray in color. *Bone marrow of femur.*—Pale pinkish yellow. *Other organs.*—Pericardium and heart show no abnormalities. Liver and kidneys show cloudy swelling.

Cultures.—Heart's blood, left lower lobe, and spleen, Pneumococcus Type I.

Microscopic Examination.—Left lower lobe.—The pleura is edematous and infiltrated with moderate numbers of polymorphonuclear leucocytes, large mononuclear cells, and occasional lymphocytes. There is a thin meshwork of fibrin on the surface. Sections from the consolidated portion of the lobe show the alveoli uniformly filled with polymorphonuclear leucocytes, moderate numbers of large mononuclear cells, and fibrin. Some contain many red blood corpuscles, others none. The leucocytes are for the most part well preserved; the large mononuclear cells frequently contain phagocytosed leucocytes. The alveolar walls show little or no leucocytic infiltration; the alveolar capillaries are engorged. The adventitia of the arteries and veins is moderately edematous and is infiltrated with many large mononuclear phagocytes and lymphocytes. A few polymorphonuclear leucocytes and an occasional plasma cell are also seen. The perivascular lymphatics are filled with similar cells. The walls of the bronchi and bronchioles are infiltrated with large mononuclear cells, lymphocytes, and moderate numbers of polymorphonuclear leucocytes, their lymphatics being filled with similar cells. The epithelium appears intact; the lumina contain a variable amount of exudate consisting of leucocytes, red blood corpuscles, and fibrin. Sections at the margin of the consolidated area show the alveoli irregularly filled with exudate (Fig. 25). In some, large mononuclear phagocytic cells predominate, in others, the exudate consists almost entirely of red blood corpuscles, while in others, polymorphonuclear leucocytes are abundant. Many contain coagulated serum and fibrin with comparatively little cellular exudate. The alveolar capillaries are intensely engorged. Surrounding this area is a zone in which the alveoli contain only serum and occasional desquamated epithelial cells. The alveolar walls show some infiltration with polymorphonuclear leucocytes and large mononuclear cells. The perivascular tissue is conspicuously infiltrated. The bronchioles contain little or no exudate. Few pneumococci are seen in the consolidated portion of the lobe and, for the most part, they stain poorly and are contained within polymorphonuclear leucocytes of the alveolar exudate. None are seen in the perivascular or peribronchial tissue. At the margins of the consolidated area, however, they are quite numerous in the alveolar exudate and stain well. In the surrounding zone of edematous alveoli they are present both in the serous exudate and in the alveolar walls (Fig. 26); while in the anterior portions of the lobe, where exudation into the alveoli has not occurred, they are seen only in the alveolar walls lying between the capillary and the epithelium lining the alveoli. *Bronchial lymph nodes.*—The marginal lymph sinuses are filled with numerous large mononuclear cells, many of which show inclusions of polymorphonuclear leucocytes and red blood corpuscles. Moderate numbers of lymphocytes, polymorphonuclear leucocytes, and red blood corpuscles are also present (Fig. 33). *Bone marrow of femur.*—Largely devoid of blood cells (Fig. 34). A few myelocytes, lymphocytes, immature polymorphonuclear leucocytes, and megaloblasts are scattered through the tissue or aggregated in small clumps. Groups of normoblasts and an occasional megalocaryocyte are also seen.

Protocol 3.—Monkey 26. *Macacus syrichtus*, male; weight 2,680 gm. Mar. 21, 1919. Intratracheal injection of 1 cc. of Pneumococcus Type I. Developed pneumonia. Killed on 9th day while still sick. Leucocytosis at time of death.

Anatomical Diagnosis.—Lobar pneumonia, right and left lower lobes; stage of gray hepatization; acute fibrinous pleuritis, bilateral.

Left pleural cavity.—Contains no fluid; there are fresh fibrinous adhesions between the visceral and parietal pleuræ over the left lower lobe. *Right pleural cavity.*—Contains no fluid; there is a fresh fibrinous exudate on the posterior and external surfaces of the right lower lobe. *Left lung.*—There is a massive and uniform consolidation of the left lower lobe involving the entire lobe except the anterior margin which is crepitant. The cut surface is uniformly yellowish gray, finely granular, and comparatively dry. The bronchi and bronchioles are not prominent, but contain a moderate amount of exudate. The upper and middle lobes are collapsed, pale pink, and crepitant throughout. *Right lung.*—The lower lobe shows a massive consolidation similar in all respects to that on the left. The cut surface presents the typical appearance of gray hepatization. *Bronchial lymph nodes.*—Enlarged and succulent. *Bone marrow of femur.*—Of normal consistency; yellowish pink in color. *Other organs.*—Pericardium normal. Heart appears moderately dilated, but otherwise normal. Liver and kidneys show cloudy swelling.

Cultures.—Heart's blood and lungs, no growth; bronchus, Pneumococcus Type I.

Microscopic Examination.—Left lower lobe.—The pleura is thickened and infiltrated with large numbers of polymorphonuclear leucocytes. The lining pleural cells are well preserved. The pleura is covered with a dense meshwork of fibrin infiltrated with leucocytes. The alveoli, except at the extreme margins of the lobe, are uniformly filled with an exudate of polymorphonuclear leucocytes and fibrin (Fig. 29). A few large mononuclear phagocytic cells and lymphocytes are also seen. The alveolar walls are thin and show little or no leucocytic infiltration. The alveolar capillaries are compressed and empty. The leucocytes of the alveolar exudate are for the most part well preserved, but in portions of the lobe the alveolar exudate is undergoing disintegration, and there is considerable proliferation and desquamation of alveolar epithelium. The adventitia of the vessels, though not distended with edema, is infiltrated with polymorphonuclear leucocytes and moderate numbers of large mononuclear cells and lymphocytes. There is no new connective tissue formation. The walls of the bronchi and bronchioles do not appear thickened, but are everywhere infiltrated with a few polymorphonuclear leucocytes, large mononuclear phagocytes, and lymphocytes. The epithelium is well preserved except for occasional places where desquamation has occurred. The alveolar ducts, terminal bronchioles, and bronchi are filled with an exudate of leucocytes and fibrin. The perivascular and peribronchial lymphatics, though not conspicuously distended, contain many polymorphonuclear leucocytes and moderate numbers of large

mononuclear cells and lymphocytes. The interlobular septa are not conspicuous. In the peripheral alveoli near the pleura the exudate is less compact and frequently shows a preponderance of large mononuclear phagocytic cells with inclusions of leucocytes. The alveolar walls are greatly thickened by infiltration with polymorphonuclear leucocytes. The perivascular tissue is conspicuous and shows a dense leucocytic infiltration, the perivascular lymphatics being filled with leucocytes. Pneumococci are very scanty in the alveolar exudate and when seen stain poorly or are phagocytosed. In the anterior margin where consolidation has not occurred many are seen in the alveolar walls or in the coagulated serum in the alveoli. *Bone marrow of femur.*—Shows a marked cellular hyperplasia. All types of hematopoietic cells are present in abundance, mature forms being very numerous (Fig. 35).

Protocol 4.—Monkey 89. *Macacus syrichtus*, male; weight 2,462 gm. May 20, 1919. Intratracheal injection of 0.001 cc. of Pneumococcus Type I. Developed pneumonia and recovered by crisis on May 31. June 4. Killed.

Anatomical Diagnosis.—Lobar pneumonia, right lower lobe; stage of resolution; organizing pleuritis, right.

Left pleural cavity.—Contains no fluid; pleural surfaces glistening. *Right pleural cavity.*—Contains no fluid; the lower portion is obliterated by moderately firm adhesions between the parietal and visceral pleuræ. *Left lung.*—Collapsed, pale pink, and crepitant throughout. *Right lung.*—The lower lobe presents a translucent gray appearance. It is moderately firm throughout but of a doughy consistency. The cut surface presents a fairly uniform pinkish gray gelatinous appearance. The bronchi contain a small amount of mucoid material. The upper and middle lobes are pale pink and crepitant throughout.

Cultures.—Heart's blood and right lower lobe, no growth.

Microscopic Examination.—Right lower lobe.—The pleura is covered with a layer of moderately vascular granulation tissue, the interstices of which are infiltrated with numbers of lymphocytes and occasional large mononuclear cells, polymorphonuclear leucocytes, and plasma cells. The pleural lymphatics are filled with similar cells. The alveoli are almost uniformly filled with a pink-staining finely granular material (Fig. 30). The majority contain many large round or oval mononuclear cells with faint staining, sometimes vacuolated protoplasm. A few of these cells contain inclusions of polymorphonuclear leucocytes or red blood corpuscles. Occasional larger multinucleated cells of similar structure are seen. Moderate numbers of lymphocytes are present and an occasional polymorphonuclear leucocyte or red blood corpuscle is also seen. The alveolar epithelium shows active proliferation in many places. The alveolar walls are not infiltrated, their capillaries not engorged. An occasional alveolus contains a plug of organizing exudate. The adventitia of the vessels is conspicuously infiltrated with lymphocytes, moderate numbers of plasma cells, and occasional polymorphonuclear leucocytes. There is a moderate degree of new connective tissue formation about most of the vessels. The walls of the bronchi

and bronchioles are similarly though less conspicuously infiltrated. The lumina contain a small amount of granular material and a few large mononuclear cells similar to those in the alveoli. No pneumococci are seen.

Protocol 5.—Monkey 64. *Macacus syrichtus*, female; weight 2,600 gm. May 6, 1919. Intratracheal injection of 0.000001 cc. of Pneumococcus Type I. Developed pneumonia; improving May 13, but continued to run an irregular fever until June 2. June 4. Appeared well. Killed.

Anatomical Diagnosis.—Lobar pneumonia, left lower lobe; organization; fibrous pleuritis, left.

Left pleural cavity.—Contains no fluid; the lower portion is obliterated by moderately firm fibrous adhesions. *Right pleural cavity.*—Contains no fluid; pleural surfaces glistening. *Left lung.*—The lower lobe is firmly consolidated throughout, of translucent gray color, and rubber-like consistency. The cut surface is light gray, dry, and studded with fine, firm granulations. The vessels, bronchi, and interlobular septa are prominent. The upper and middle lobes are pale pink and crepitant. *Right lung.*—Voluminous, pale pink, and crepitant throughout.

Cultures.—Heart's blood, no growth; left lower lobe, four colonies of Pneumococcus Type I; left main bronchus, Pneumococcus Type I.

Microscopic Examination.—Left lower lobe.—The peribronchial and perivascular tissue and the interlobular septa are everywhere greatly thickened by the formation of new connective tissue, the interstices of which are infiltrated with many lymphocytes and plasma cells. In places polymorphonuclear leucocytes are also present. The alveolar walls are similarly affected in many parts of the section, in other places they show no organization. In some areas the process of organization is so extensive that only the distorted and compressed outlines of the alveoli remain. Organization and resolution of the alveolar exudate are going on side by side in adjacent alveoli (Fig. 31). Some alveoli contain numerous desquamated epithelial cells, together with a few lymphocytes and occasional polymorphonuclear leucocytes, in a groundwork of amorphous material. The alveolar epithelium shows very active and widespread proliferation. Other alveoli are filled with plugs of new connective tissue infiltrated with lymphocytes and plasma cells. These plugs are covered with an epithelial layer, the epithelial cells sometimes being cuboidal, sometimes thin and flattened out. A few small groups of alveoli filled with polymorphonuclear leucocytes are also seen. The alveolar ducts and many of the terminal bronchioles are filled with masses of new connective tissue similar to those in the alveoli. The bronchial epithelium appears intact. The bronchi contain polymorphonuclear leucocytes, lymphocytes, and desquamated alveolar epithelial cells in a groundwork of amorphous material.

Pathogenesis of Lobar Pneumonia.

Although the pathology of lobar pneumonia has been well known for many years, little definite knowledge concerning the pathogenesis of the disease has developed. Two principal theories with respect to the initial mode of infection have existed. Some writers[3] have advocated the theory that lobar pneumonia is hematogenous in origin, basing this belief on the fact that pneumococci have been isolated from the blood prior to the development of symptoms or physical signs of pneumonia. This point of view, however, has received no experimental support, since attempts to produce lobar pneumonia in animals by intravenous inoculation of pneumococci have consistently failed.[4, 5, 6] On the other hand, the more commonly accepted view that the mode of infection is by way of the air passages has received a certain amount of confirmation in the experimental production of pneumococcus pneumonia in animals by various methods of intratracheal or intrabronchial inoculation.[1, 6–9] However, these experiments have not been conclusive, either because the pneumonia produced was not clearly lobar pneumonia or because the method of inoculation or amount of culture material injected tended to vitiate the value of the experiments.

Knowledge concerning the initial point of invasion of the lung by the pneumococcus, the character and location of the primary lesions, and the method by which the infection spreads throughout the lung would appear to be equally doubtful, if one may judge by the indefiniteness with which these subjects are presented in standard textbooks of pathology. Even in the later stages, although it is generally recognized that hepatization in lobar pneumonia is a progressive process, there still remains a difference of opinion as to whether consolidation begins at the periphery and extends centrally or *vice versa*.

[3] Kidd, P., *Lancet*, 1912, i, 1589.
[4] Wadsworth, A., *Am. J. Med. Sc.*, 1904, cxxvii, 851.
[5] Rasquin, E., *Arch. méd. exp. et anat. path.*, 1910, xxii, 804.
[6] Armstrong, R. R., *Brit. Med. J.*, 1914, ii, suppl., 57.
[7] Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.
[8] Wollstein, M., and Meltzer, S. J., *J. Exp. Med.*, 1912, xvi, 126.
[9] Winternitz, M. C., and Hirschfelder, A. D., *J. Exp. Med.*, 1913, xvii, 657.

Review of the literature reveals a striking paucity of attempts to answer these questions by experimental procedures. Papers by Müller[10] and by Rasquin[5] are of particular interest. Müller undertook a study of the pathogenesis of aspiration pneumonia experimentally produced in rabbits by vagotomy. He showed that bacteria-bearing material aspirated from the mouth had penetrated as far as the terminal bronchioles by the end of 6 hours. By 8 hours evidence of bacterial infection was present. The walls of alveoli lying adjacent to the alveolar ducts in which affected bronchioles terminated were swollen and infiltrated with leucocytes, and there were epithelial desquamation and beginning exudation into these alveoli, but the alveolar ducts, atria, and alveoli to which the affected bronchioles led were free from exudate. Bacteria were seen in the infiltrated alveolar walls and in the adjacent alveoli, but not in those with which the affected terminal bronchioles communicated. Still later with wider extension of the process the bacteria were found spreading in the lymph channels of the interstitial framework of the lung and in the alveolar walls, exudation and passage of bacteria into the alveolar spaces occurring only after bacterial invasion of the alveolar walls had occurred. From these observations he inferred that the bacteria gained entrance into the pulmonary tissue at the point where the cuboidal epithelium of the terminal bronchiole gives place to the flattened epithelium of the alveolar duct and atrium, and that the invasion was facilitated by the mechanical injury caused by the aspirated foreign material. He established the fact that further spread of the infection was by way of the interstitial tissue of the lung framework and by way of the alveolar walls.

Rasquin showed that in pneumonia experimentally produced in rabbits by the intratracheal injection of attenuated pneumococci, consolidation invariably occurred in the portions of the lobe proximal to the hilum and usually in the posterior half, the distal portions being free from consolidation. Since the rabbits died with pneumococcus septicemia in a comparatively short time complete lobar consolidation was not found and he considered the process comparable with catarrhal rather than with lobar pneumonia.

In the present study of lobar pneumonia experimentally produced in monkeys by the intratracheal injection of pneumococcus, the attempt has been made to determine the following points concerning the pathogenesis of the disease: (1) whether lobar pneumonia is a bronchiogenic or a hematogenous infection; (2) at what point or points the pneumococcus primarily invades the lung tissue; (3) what the character and location of the initial lesions are; (4) by what paths the pneumococci spread from the initial points of invasion to the remainder of the lobe; and (5) whether hepatization begins more or less simultaneously throughout a lobe or whether it begins centrally and spreads peripherally, or *vice versa*.

[10] Müller, W., *Deutsch. Arch. klin. Med.*, 1902, lxxiv, 80.

Initial Path of Infection.—It has been stated in the preceding paper[1] that pneumonia was consistently produced in normal monkeys by the intratracheal injection of pneumococcus, and it was shown that the pneumonia so produced ran a clinical course identical with that of lobar pneumonia in man. It was furthermore stated that attempts to produce pneumonia by subcutaneous or intravenous inoculation consistently failed. This was equally true of normal monkeys and of monkeys whose resistance to pneumococcus infection had been increased by preliminary inoculations with pneumococcus vaccine. It was also shown that pneumococci may enter the blood stream following intratracheal injection, in some instances before the symptoms or physical signs of pneumonia have developed. It therefore seems safe to conclude that lobar pneumonia is a bronchiogenic and not a hematogenous infection.

Site of Primary Invasion of the Lung Tissue.—It is conceivable that the pneumococcus may primarily invade the lung tissue early in the course of the bronchi by penetration of the bronchial mucous membrane, or that it may pass down the lumina of the bronchi to the terminal bronchioles, and there penetrate the epithelium, or that it may be carried still further and invade the lung tissue only after it has gained access to the alveolar ducts, atria, or alveoli. In an attempt to answer this question a monkey was injected intratracheally with the growth in 10 cc. of an 18 hour broth culture of pneumococcus thrown down by centrifuge and resuspended in 1.5 cc. of the supernatant broth. 3 hours after injection the monkey was killed and autopsy immediately performed. The protocol follows.

Protocol 6.—Monkey 72. *Macacus syrichtus*, female. Apr. 23, 1919, 11 a.m. Intratracheal injection of 10 cc. (in 1.5 cc.) of 18 hour broth culture of Pneumococcus Type I. 2 p.m. Killed.

Autopsy.—Pleural cavities.—Contain no fluid; pleural surfaces glistening. *Left lung.*—Pale pink and air-containing throughout. No edema or congestion. *Right lung.*—The lower lobe shows an edematous appearing area at the root, the cut surface yielding a moderate amount of fluid. There is no congestion. The remainder of the lobe and the upper and middle lobes appear normal. The larger bronchi contain a moderate amount of frothy fluid. The smaller bronchi are empty. *Lymph nodes.*—Nodes at the hilum appear moderately enlarged. *Other organs.*—Show nothing of importance.

Bacteriology.—Stained films from trachea and right main bronchus show many pneumococci; the bronchial exudate also shows moderate numbers of polymor-

phonuclear leucocytes. There is no phagocytosis. *Cultures.*—Heart's blood, Pneumococcus Type I; trachea, Pneumococcus Type I; right main bronchus, Pneumococcus Type I; left main bronchus, no growth; small bronchi in right and left lower lobes, no growth.

Microscopic Examination.—Right lower lobe.—In sections cut close to the hilum the perivascular and peribronchial tissue is edematous and infiltrated with polymorphonuclear leucocytes. The interlobular septa and portions of the pleura are similarly affected. The walls of the smaller bronchi and terminal bronchioles and of their accompanying arteries are densely infiltrated with leucocytes. The walls of the alveolar ducts, atria, and adjacent alveoli are likewise infiltrated (Fig. 22). The epithelium of the large bronchi shows active secretion of mucus but otherwise appears intact in most places. At a number of points, however, the epithelium is injured, and here many polymorphonuclear leucocytes are seen passing into the lumen of the bronchus where they are accumulated in masses on the surface. The bronchioles, alveolar ducts, and atria contain no exudate except in a few instances where the process is well advanced. The alveoli are free from exudate and often appear atelectatic. Moderate numbers of pneumococci are seen entangled in the cilia of the bronchial epithelium or in the mucus secretion. At the points where the bronchial epithelium shows injury pneumococci are seen penetrating the bronchial wall (Fig. 19). Small numbers of pneumococci are seen in the margins of the peribronchial and perivascular tissue, apparently largely in the lymphatics. Pneumococci are more numerous in the walls of the smaller bronchi and vessels, and some are seen in the interlobular septa and affected portions of the pleura. They are present in considerable numbers in the walls of the terminal bronchioles, alveolar ducts, and atria. In the alveolar walls lying adjacent to the vessels, the interlobular septa, and especially the alveolar ducts, they are very abundant, apparently lying between the capillaries and the lining epithelium. None are seen in the lumina of the terminal bronchioles, alveolar ducts, or alveoli, except in a few places where the lesion is well advanced and exudation into the alveoli or alveolar ducts has occurred. In sections from more distal portions of the lobe the tissue appears normal and no pneumococci are seen. *Right upper lobe.*—Sections adjacent to the hilum show a similar picture to that found in the lower lobe. *Other lobes.*—No abnormalities are shown and no pneumococci are seen. *Bronchial lymph node.*—The marginal sinus is crowded with large numbers of polymorphonuclear leucocytes and a few large mononuclear cells and lymphocytes. An occasional free or phagocytosed pneumococcus is seen. *Trachea.*—Appears normal except for a moderately excessive mucus secretion. A few pneumococci are seen entangled in the cilia of the epithelium.

In attempting to form any certain opinion from the results of this experiment as to where the pneumococcus primarily invades the lung tissue, it is obvious that a considerable degree of conservatism is

necessary, since the number of pneumococci injected was very large and since considerable invasion of the tissue had already occurred at the time the animal was killed. It at least may be stated with reasonable positiveness that the initial invasion occurs somewhere comparatively close to the hilum. That it occurred in this case, in part at least, by direct penetration of pneumococci into the walls of the large bronchi is clear. Whether pneumococci had passed down the lumina of the bronchi to the terminal bronchioles or even further into the alveolar ducts and alveoli of the portion of the lobe proximal to the hilum and there invaded the lung tissue is uncertain. It seems doubtful, however, in view of the fact that no pneumococci were found in the lumina of the bronchioles, alveolar ducts, and alveoli, except in a few places where the lesion was well advanced, while on the other hand they were very numerous in the walls of these structures where the process was early and no exudation into the bronchioles or alveoli had occurred. That this practical absence of pneumococci in the terminal bronchioles and alveoli was found in spite of the very large numbers of pneumococci injected lends confirmation to this doubt. The evidence, though strongly against the latter mode of invasion, does not, however, certainly exclude it.

Character and Location of the Initial Lesions.—Opportunity to study the earliest lesions of lobar pneumonia before hepatization had begun was presented in Monkeys 17, 88, 98, 101, 109, and 114, all of which died in the stage of engorgement. The protocol of Monkey 98 has been presented above and is representative of the group. Since all these monkeys, except Monkey 17, were inoculated with minute amounts of pneumococcus culture, such as may be assumed to be reasonably comparable with the probable infecting dose in man, the picture encountered may safely be considered to be that of the earliest stages of lobar pneumonia in man.

Histological sections from all these animals showed clearly that the primary lesions were of the interstitial framework of the lung with accompanying involvement of the lymphatic system. These lesions have been presented in detail above and need no further description. The earliest alveolar lesions beside capillary engorgement were likewise interstitial in character, consisting in leucocytic infiltration of the alveolar walls before exudation into the alveoli

occurred. This took place primarily in the walls of alveoli lying adjacent to terminal bronchioles and alveolar ducts of the central portions of a lobe and to a variable extent in the walls of alveoli lying adjacent to the larger bronchi and vessels.

That the primary lesion of lobar pneumonia is one of the interstitial framework of the lung was well illustrated in Monkeys 96 and 107, which had abortive attacks with recovery on the 3rd and 4th days respectively. Autopsies showed a purely interstitial pneumonia without further advance of the process to the stage of hepatization (Fig. 10).

Spread of the Pneumococci from the Initial Points of Invasion to the Remainder of the Lobe.—This point was readily determined by study of the distribution of pneumococci in the lungs of monkeys dying in the stage of engorgement. As described in the protocols of Monkeys 98 and 72, pneumococci were found in the interstitial tissue and lymphatics of the lung, but were not present in the lumina of the bronchioles, alveolar ducts, or atria, or in the alveolar spaces. In detail, they were present in the perivascular tissue and bronchial walls (Figs. 20 and 21), in the interlobular septa and pleura, in the walls of the terminal bronchioles and alveolar ducts, and in the walls of alveoli lying adjacent to these structures. In more advanced cases of pneumonia in which partial hepatization of a lobe had occurred, a similar interstitial distribution of pneumococci was found in the peripheral unconsolidated portions (Fig. 24). Where beginning exudation had occurred into the alveoli pneumococci were found both in the alveolar walls and in the exudate (Fig. 26). Where hepatization was fully developed they were present in variable numbers in the exudate, often largely phagocytosed or staining poorly, and they were no longer present in the alveolar walls.

It is quite clear, therefore, that invasion and spread of pneumococci in the lung in lobar pneumonia is by way of the perivascular, peribronchial, and septal tissue and lymphatics, and that the alveolar structure is primarily infected by spread of the pneumococci from the grosser framework of the lungs into the alveolar walls. With further advance of the process to the stage of hepatization pneumococci pass out from the alveolar walls into the alveolar spaces together with the exudate.

Development of Hepatization.—It has been stated above and illustrated by the protocol of Monkey 23 that in monkeys which died early in the stage of hepatization the consolidated areas were found invariably to occupy the portions of the lobe proximal to the hilum, while the more distal portions of the lobe were still air-containing (Fig. 8). Although by no means an absolute rule, hepatization usually occurred first in the posterior and upper parts of the lower lobes, in the lower parts of the middle lobes, and in the lower and posterior parts of the upper lobes. This distribution in no way precluded early extension of the consolidation as far as the pleura, since the pleura is comparatively near the large bronchi and vessels in the part of the lobe proximal to the hilum. As the disease progressed hepatization spread to the distal portions of the lobe until complete or nearly complete lobar consolidation had occurred (Fig. 9). As in lobar pneumonia in man, the anterior margins frequently remained air-containing.

Whether a definite lobular arrangement of the exudate might be recognized in the early stages of hepatization has been impossible to determine. It seems possible that a certain amount of variation exists in this respect, depending upon the rapidity with which pneumococci are distributed throughout the lung and the rapidity with which hepatization develops.

Although it seems reasonable to conclude from the study of the development of hepatization in lobar pneumonia in monkeys that consolidation in lobar pneumonia in man begins centrally and spreads peripherally, this does not preclude the probability that in a rapidly spreading pneumococcus invasion of the lung hepatization of an entire lobe may occur within a comparatively short time, nor does it preclude the possibility that in fairly resistant individuals the infection might at first be limited to the interstitial framework in the central portions of the lobe with the primary development of hepatization in the peripheral portions where the framework is less dense. In the latter case, the picture would approach that of a lobular pneumonia or at most a lobar pneumonia of limited involvement.

SUMMARY.

Study of the pathology of pneumonia experimentally produced in monkeys by the intratracheal injection of pneumococcus has shown that it is identical with the pathology of lobar pneumonia in man. It has been found that the pneumococcus primarily invades the pulmonary tissue at some point or points in the portion of the lobe proximal to the hilum, that it spreads rapidly throughout the lobe by way of the perivascular, peribronchial, and septal interstitial tissue and lymphatics, quickly reaching the pleura, and that it invades the alveolar structure primarily by way of the alveolar walls, subsequently passing into the alveolar spaces simultaneously with the outpouring of exudate into the alveoli. It has been shown that the initial mode of invasion may be by direct penetration at one or more points into the walls of the larger bronchi near the hilum. The possibility that primary invasion may occur in terminal bronchioles, alveolar ducts, or alveoli of the parenchyma near the hilum has not been certainly excluded, though the evidence is against this supposition. In harmony with the mode of distribution of pneumococci it has been found that the initial lesions of lobar pneumonia are of the interstitial framework of the lung, with respect both to the grosser framework and to the alveolar framework. Hepatization begins centrally and spreads toward the periphery and is a constantly progressive process. With the development of hepatization the conspicuous interstitial lesions of the earliest stages gradually diminish and are often largely masked when complete lobar consolidation has developed. Resolution is frequently accompanied by a varying degree of organization of the grosser framework of the lung. A variable amount of organization of the alveolar exudate also may occur.

CONCLUSIONS.

1. In lobar pneumonia the pneumococcus primarily invades the lung tissue at some point or points near the root of the lobe.

2. The pneumococcus subsequently spreads throughout the lobe by way of the interstitial framework and lymphatic systems.

3. Lobar pneumonia, therefore, is primarily an interstitial infection of the lung.

4. Consolidation in lobar pneumonia begins in the alveolar tissue proximal to the hilum and progressively spreads to the more distal tissue until complete lobar consolidation develops.

EXPLANATION OF PLATES.

PLATE 22.

FIG. 1. Monkey 83. Lobar pneumonia, Pneumococcus Type I; died on 8th day. Posterior aspect of lungs showing lobar consolidation of right middle and lower lobes, partial consolidation of right upper lobe, and normal left lung.

PLATE 23.

FIG. 2. Monkey 83. Lobar pneumonia, Pneumococcus Type I; died on 8th day. Cut surface of lungs showing red and gray hepatization of right upper and lower lobes, gray hepatization of right middle lobe, and normal left lung.

PLATE 24.

FIG. 3. Monkey 86. Lobar pneumonia, Pneumococcus Type I; died on 14th day. Cut surface of lungs showing gray hepatization of both lower lobes.

FIG. 4. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Section from right lower lobe showing thickening of vascular adventitia and of bronchial walls. × 3.5.

PLATE 25.

FIGS. 5 and 6. Monkey 88. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Sections from left lower lobe and right lower lobe, respectively, showing involvement of interstitial framework of lung. × 3.5.

PLATE 26.

FIG. 7. Monkey 112. Lobar pneumonia, Pneumococcus Type I; died on 6th day; stage of engorgement and red and gray hepatization. Section from left lower lobe showing beginning peribronchial consolidation. × 3.5.

FIG. 8. Monkey 29. Lobar pneumonia, Pneumococcus Type I; died on 2nd day; stage of engorgement and red hepatization. Section through left lower lobe showing consolidation of central portion with engorgement and edema of peripheral portions.

PLATE 27.

FIG. 9. Monkey 93. Lobar pneumonia, Pneumococcus Type I; died on 13th day; stage of gray hepatization. Section through right lower lobe showing uniform consolidation except at anterior margin. × 3.5.

FIG. 10. Monkey 107. Abortive lobar pneumonia, Pneumococcus Type III; recovered by lysis on 4th day; killed on 5th day. Section through right lower lobe showing limitation of involvement to interstitial framework of lung. × 3.5.

PLATE 28.

FIG. 11. Monkey 17. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Edema and leucocytic infiltration of adventitia of large pulmonary vein. × 100.

PLATE 29.

FIG. 12. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Edema and leucocytic infiltration of wall of large bronchus. × 100.

FIG. 13. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Perivascular edema and leucocytic infiltration of a small peripheral vein. × 100.

PLATE 30.

FIG. 14. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Leucocytic infiltration about a small vein. × 400.

PLATE 31.

FIG. 15. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Bronchiole and pulmonary arteries showing leucocytic infiltration of walls with absence of exudate in bronchiole and adjacent alveoli. × 100.

FIG. 16. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Edema and leucocytic infiltration of interlobular septum. Lymphatics filled with polymorphonuclear leucocytes. × 100.

PLATE 32.

FIG. 17. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Distended lymphatic filled with polymorphonuclear leucocytes.

FIG. 18. Monkey 98. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Capillary engorgement and atelectasis of alveoli. × 400.

PLATE 33.

FIG. 19. Monkey 72. Killed 3 hours after intratracheal injection of Pneumococcus Type I. Invasion of wall of large bronchus by pneumococci.

PLATE 34.

FIG. 20. Monkey 88. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Pneumococci in wall of large bronchus, right lower lobe. × 1,000.

FIG. 21. Monkey 88. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement. Pneumococci in perivascular tissue of small pulmonary vein near periphery. × 1,000.

PLATE 35.

FIG. 22. Monkey 72. Killed 3 hours after intratracheal injection of Pneumococcus Type I. Early alveolar lesion showing polymorphonuclear leucocytic infiltration of walls of alveolar duct and adjacent alveoli with absence of exudate in duct and alveoli. × 100.

PLATE 36.

FIG. 23. Monkey 5. Lobar pneumonia, Pneumococcus Type I; died on 1st day; stage of engorgement and red hepatization. Leucocytic infiltration of alveolar walls beyond the margin of the consolidated area; also distention of perivascular lymphatics. × 100.

PLATE 37.

FIG. 24. Monkey 6. Lobar pneumonia, Pneumococcus Type I; died on 4th day; stage of engorgement and red hepatization. Section from periphery of right lower lobe showing pneumococci in alveolar walls.

PLATE 38.

FIG. 25. Monkey 23. Lobar pneumonia, Pneumococcus Type I; died on 5th day; stage of red hepatization. Margin of consolidated area showing alveoli containing varying proportions of serum, red blood corpuscles, fibrin, polymorphonuclear leucocytes, and large mononuclear cells. × 100.

PLATE 39.

FIG. 26. Monkey 23. Lobar pneumonia, Pneumococcus Type I; died on 5th day; stage of red hepatization. Section from margin of consolidated area showing pneumococci in alveolar walls and in serous exudate in alveoli.

PLATE 40.

FIG. 27. Monkey 110. Lobar pneumonia, Pneumococcus Type I; died on 7th day; stage of red and gray hepatization. Alveolar exudate of coagulated serum, fibrin, polymorphonuclear leucocytes, and large mononuclear cells. × 400.

PLATE 41.

FIG. 28. Monkey 112. Lobar pneumonia, Pneumococcus Type I; died on 6th day; stage of red and gray hepatization. Alveolar consolidation. × 100.

FIG. 29. Monkey 26. Lobar pneumonia, Pneumococcus Type I; killed on 9th day; stage of gray hepatization. Alveoli filled with exudate of polymorphonuclear leucocytes. × 100.

PLATE 42.

FIG. 30. Monkey 89. Lobar pneumonia, Pneumococcus Type I; killed 4 days after recovery by crisis. Resolution of alveolar exudate. × 100.

FIG. 31. Monkey 64. Lobar pneumonia, Pneumococcus Type I; killed on 30th day. Organization and resolution. × 100.

PLATE 43.

FIG. 32. Monkey 14. Lobar pneumonia, Pneumococcus Type I; died on 17th day. Organization. × 100.

FIG. 33. Monkey 23. Lobar pneumonia, Pneumococcus Type I; died on 5th day. Marginal sinus of bronchial lymph node filled with large mononuclear phagocytes, polymorphonuclear leucocytes, and lymphocytes. × 400.

PLATE 44.

FIG. 34. Monkey 23. Lobar pneumonia, Pneumococcus Type I; died on 5th day. Bone marrow of femur showing almost complete exhaustion of hematopoietic cells. × 400.

FIG. 35. Monkey 26. Lobar pneumonia, Pneumococcus Type I; killed on 9th day. Bone marrow of femur showing hyperplasia. × 400.

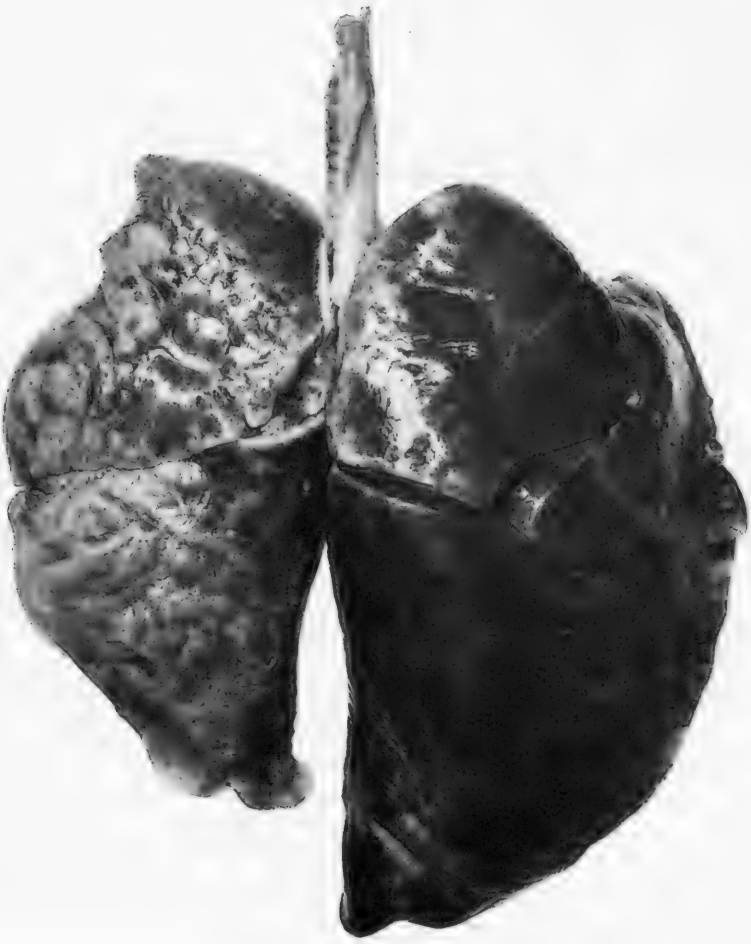

FIG. 1.

(Blake and Cecil: Experimental pneumonia. II.)

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FIG. 2.

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FIG. 3.

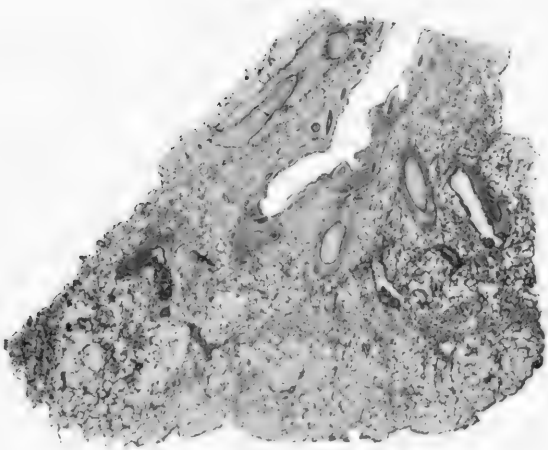

FIG. 4.

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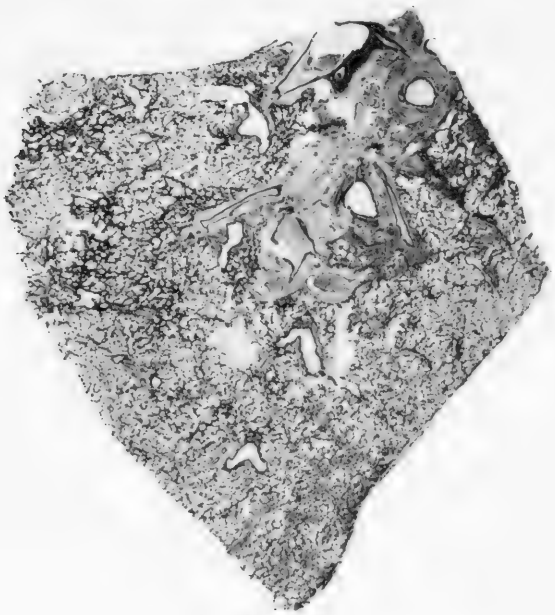

FIG. 5.

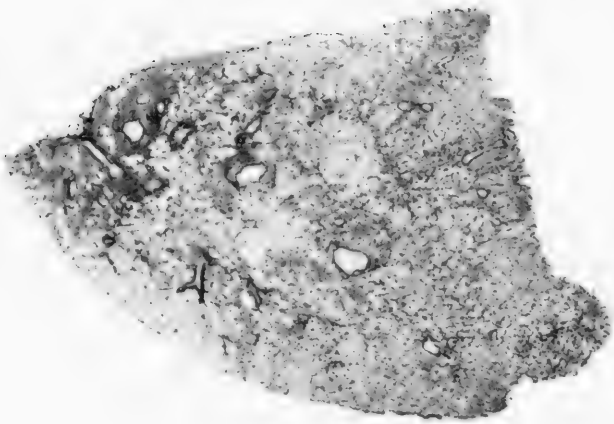

FIG. 6.

(Blake and Cecil: Experimental pneumonia. II.)

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FIG. 7.

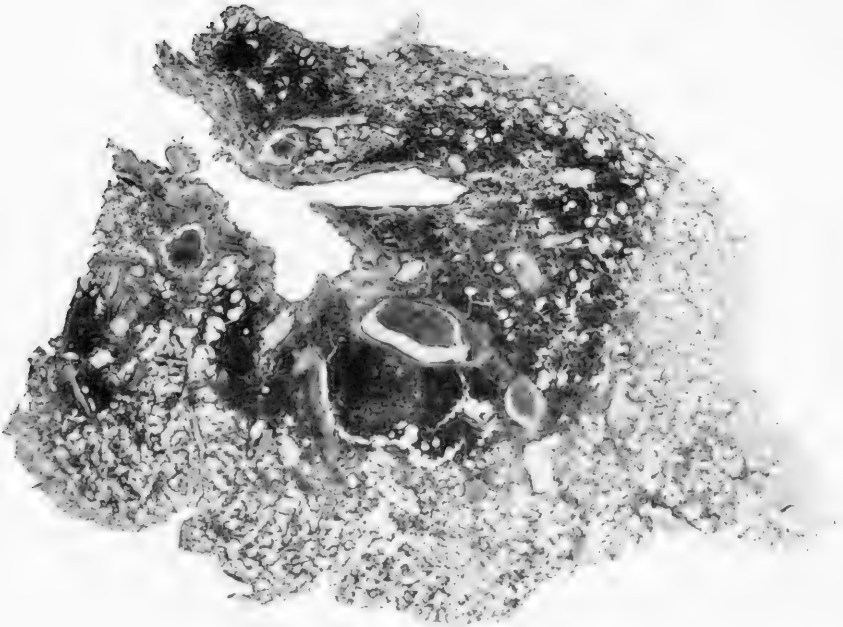

FIG. 8.

(Blake and Cecil: Experimental pneumonia. II.)

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FIG. 9.

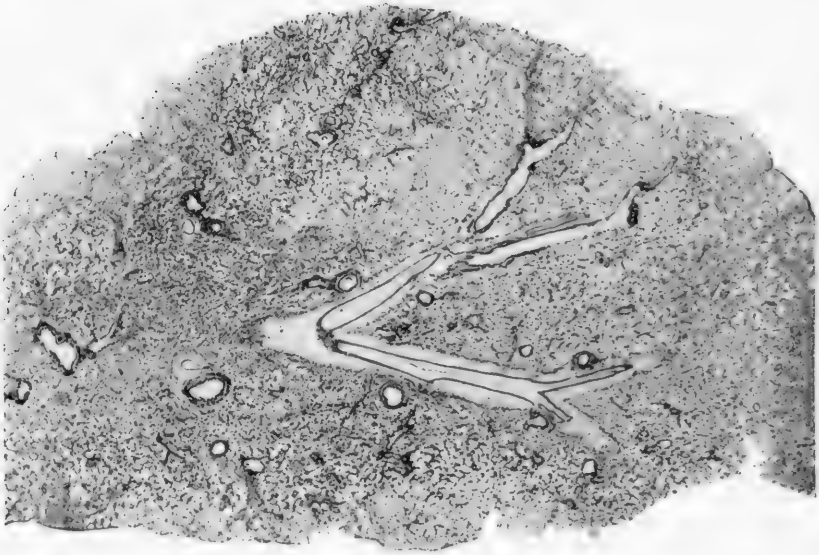

FIG. 10.

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FIG. 11.

(Blake and Cecil: Experimental pneumonia. II.)

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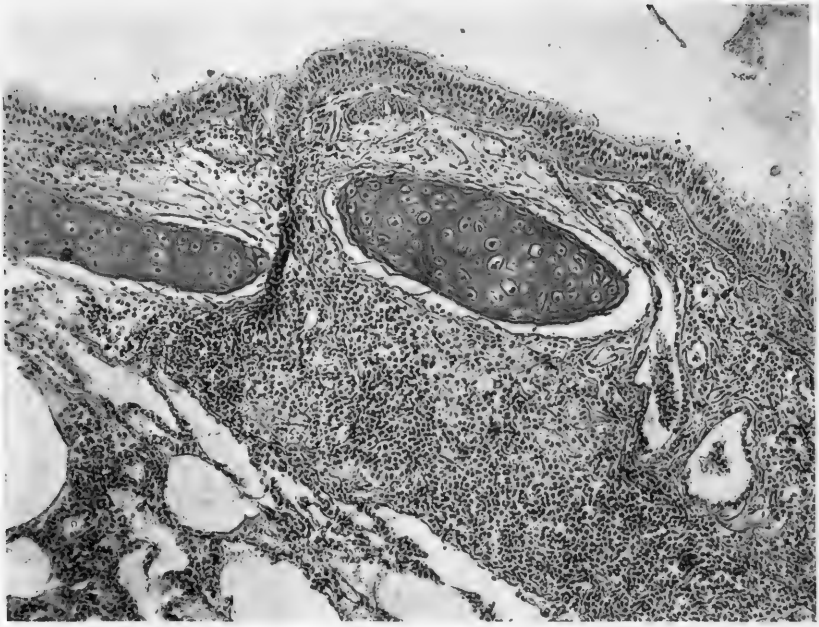

FIG. 12.

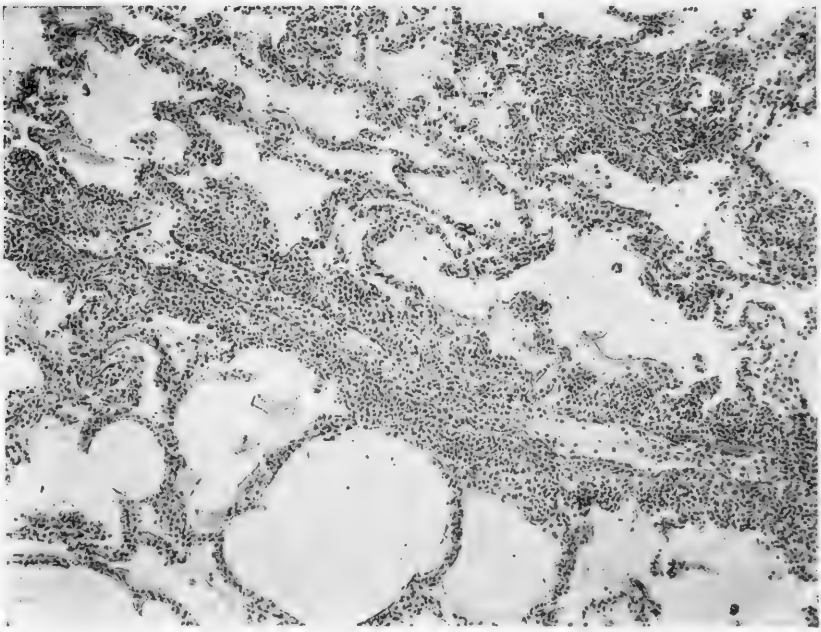

FIG. 13.

(Blake and Cecil: Experimental pneumonia. II.)

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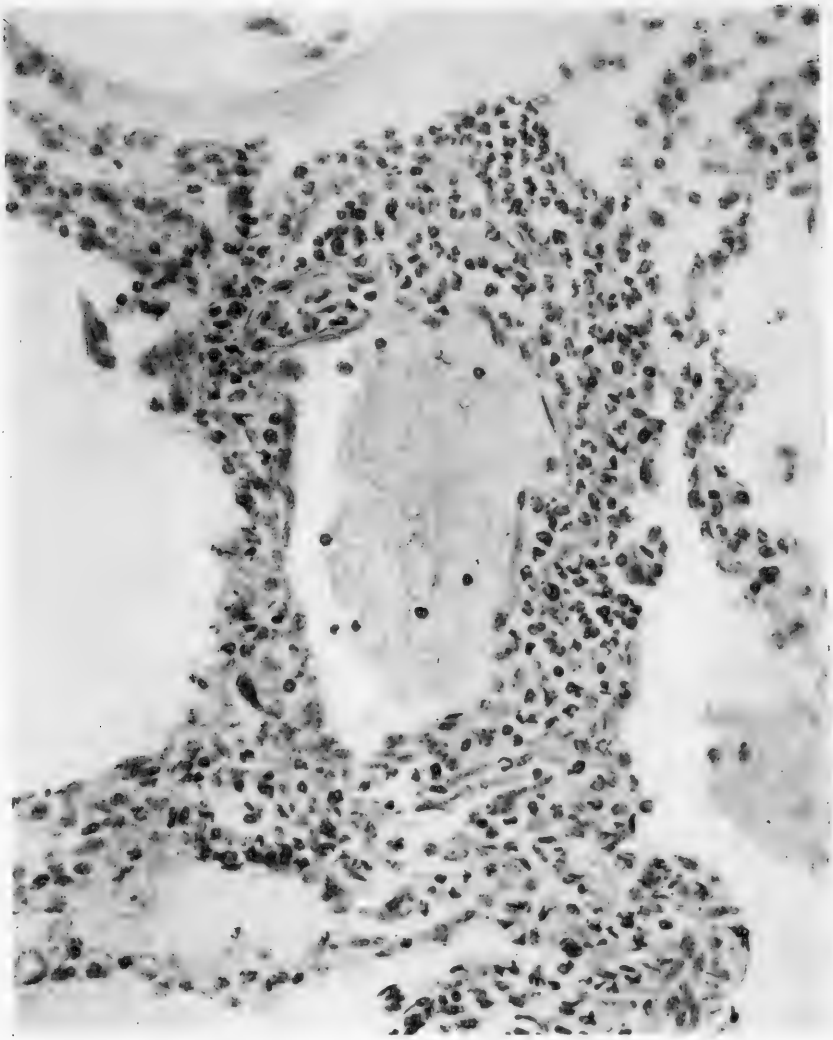

FIG. 14.

(Blake and Cecil: Experimental pneumonia. II.)

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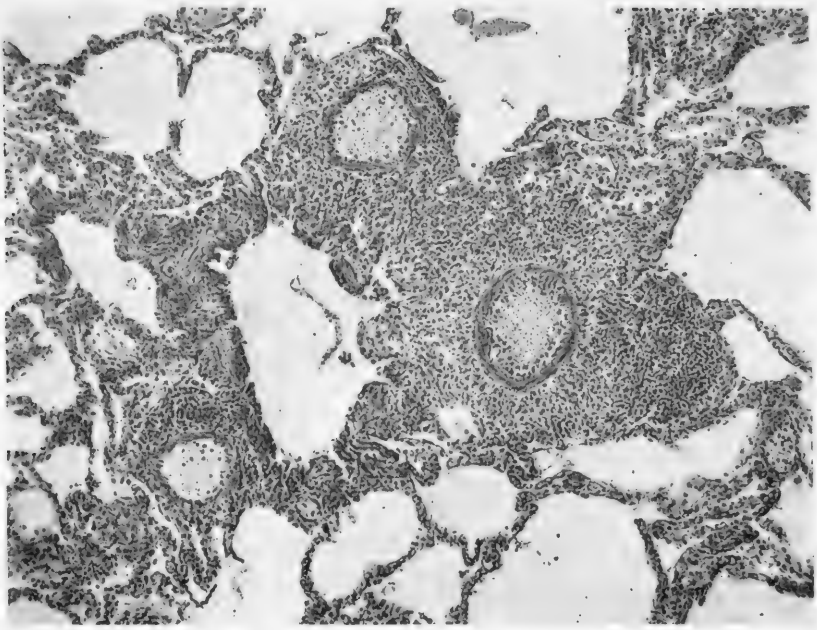

FIG. 15.

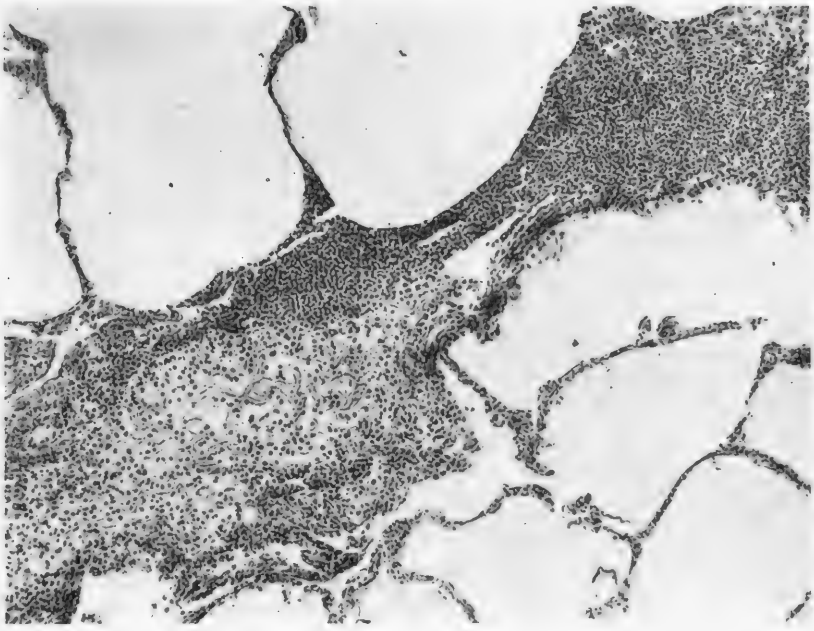

FIG. 16.

(Blake and Cecil: Experimental pneumonia. II.)

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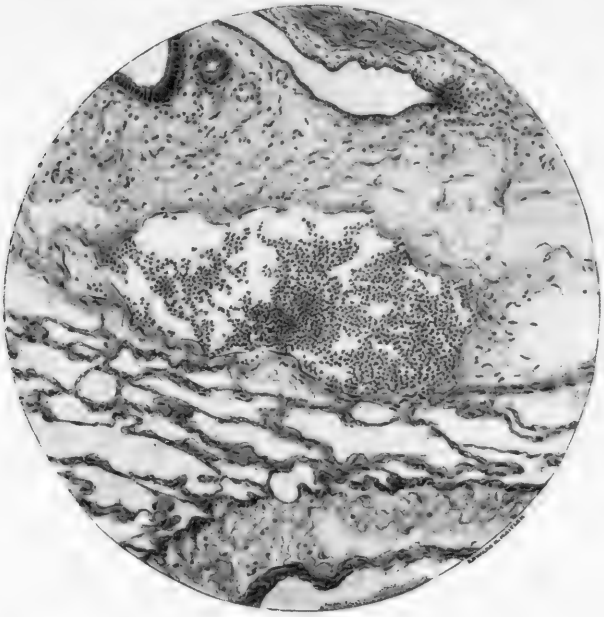

FIG. 17.

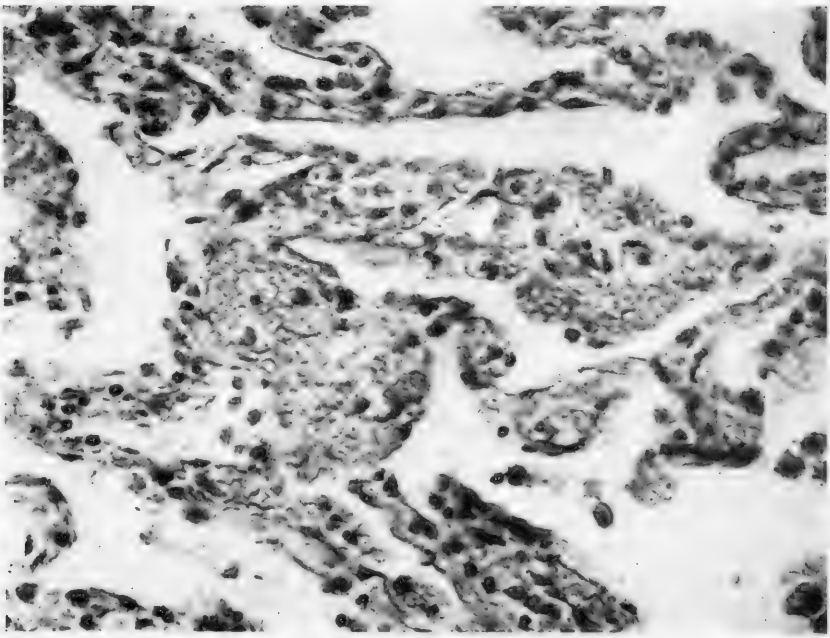

FIG. 18.

(Blake and Cecil: Experimental pneumonia. II.)

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FIG. 19.

(Blake and Cecil: Experimental pneumonia. II.)

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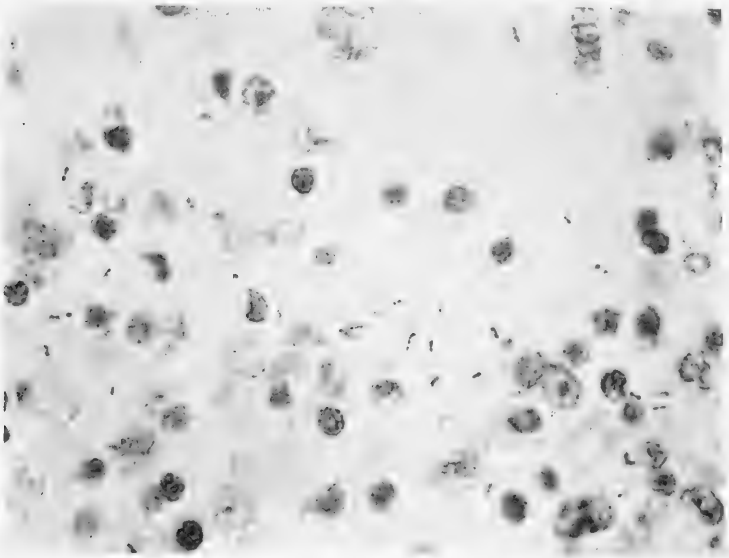

FIG. 20.

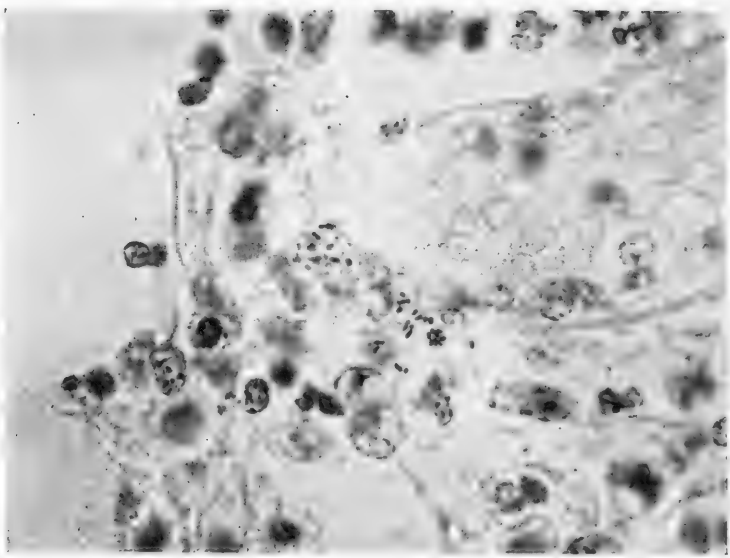

FIG. 21.

(Blake and Cecil: Experimental pneumonia. II.)

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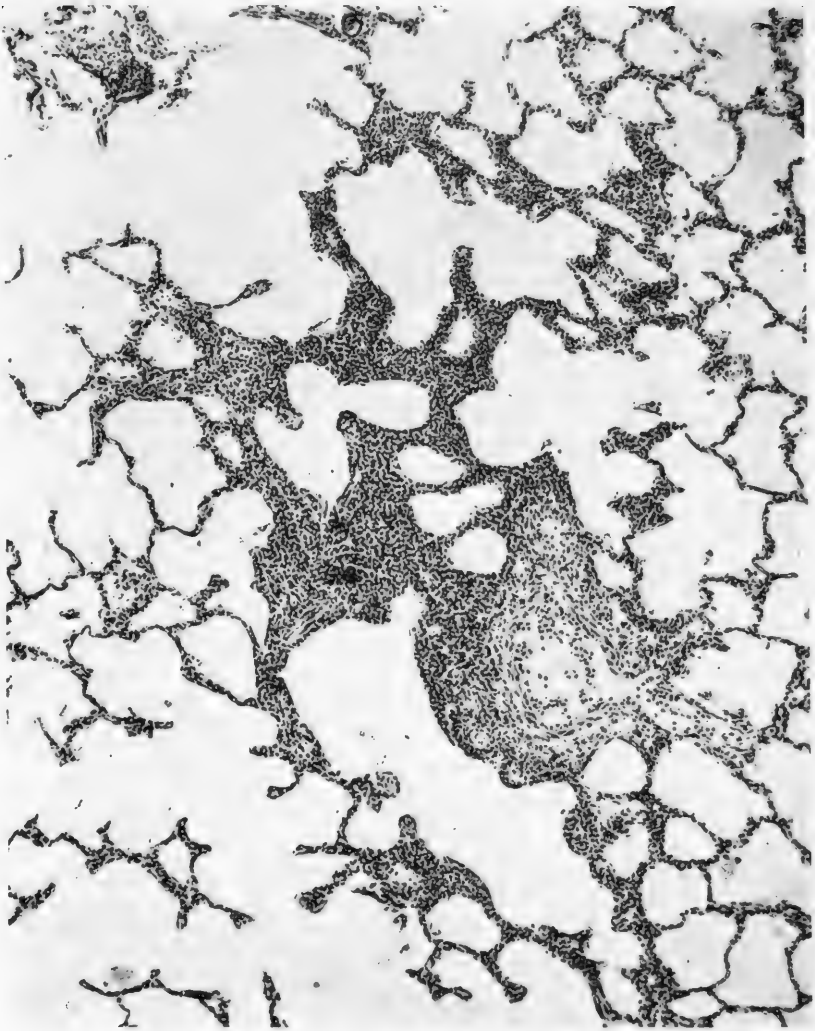

FIG. 22.

(Blake and Cecil: Experimental pneumonia. II.)

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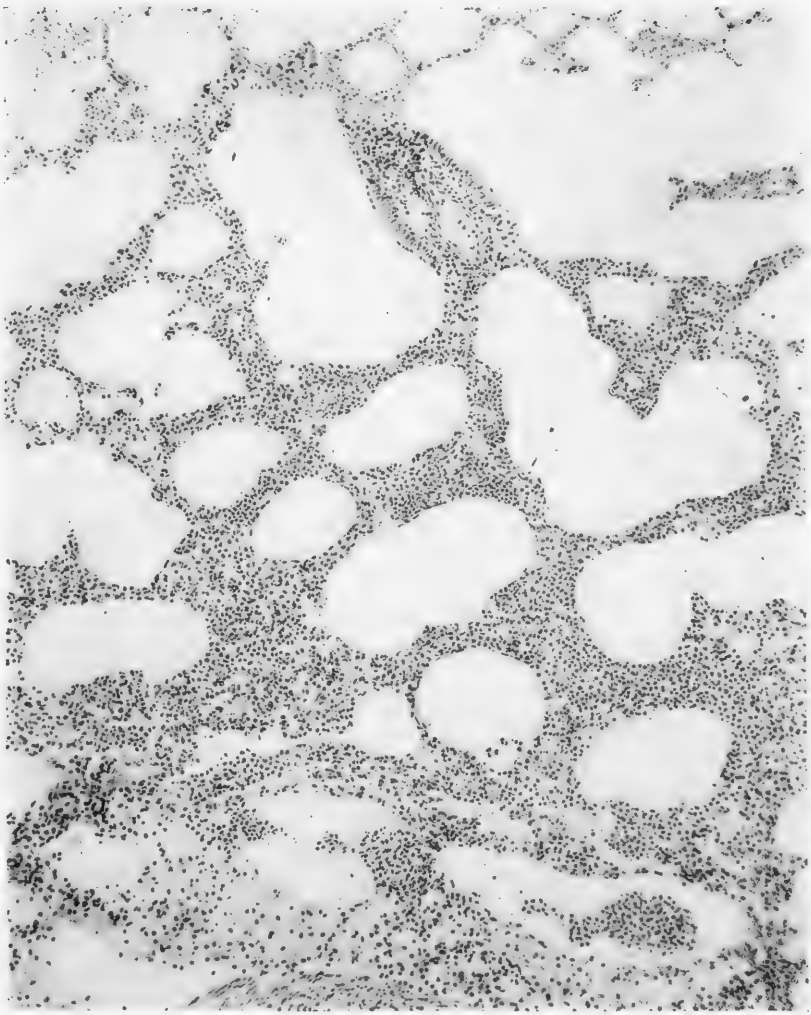

FIG. 23.

(Blake and Cecil: Experimental pneumonia. II.)

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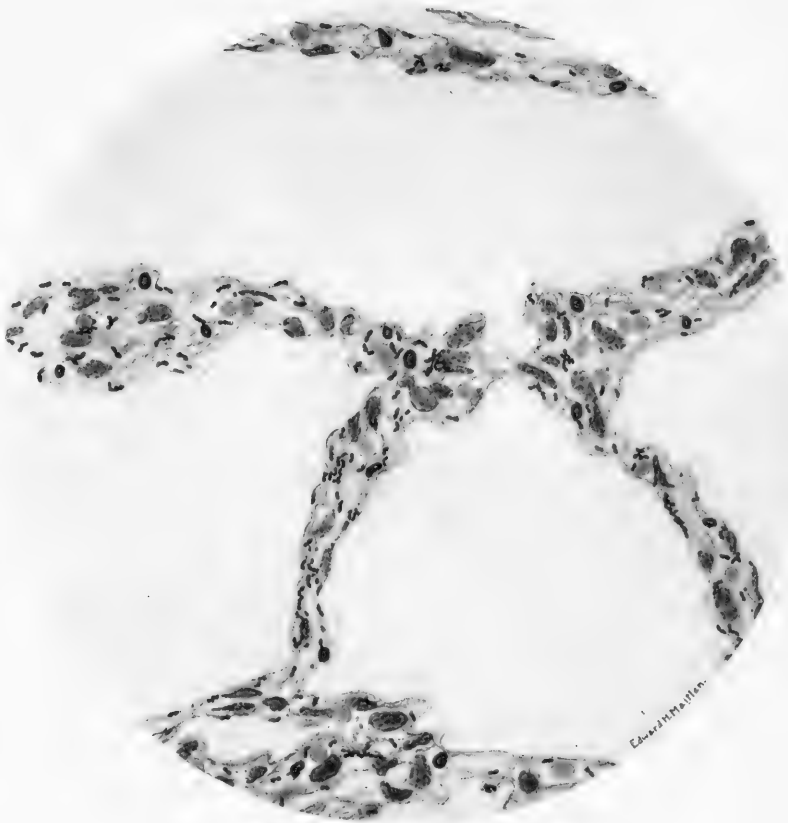

FIG. 24.

(Blake and Cecil: Experimental pneumonia. II.)

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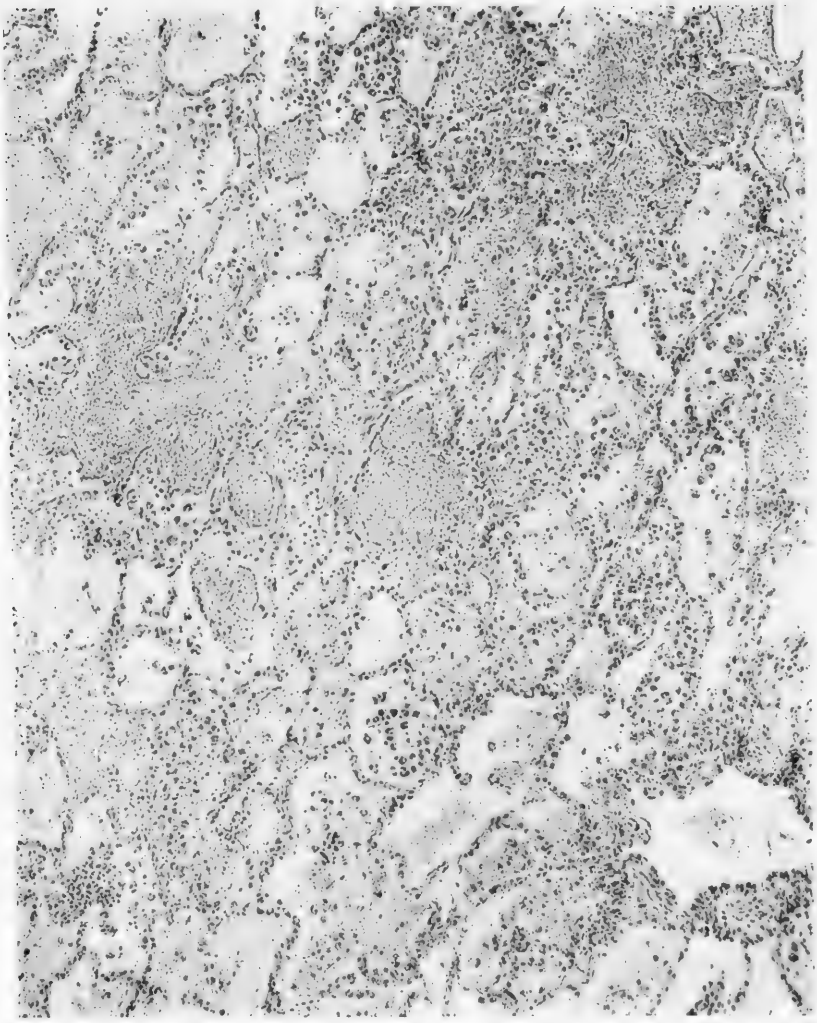

FIG. 25.

(Blake and Cecil: Experimental pneumonia. II.)

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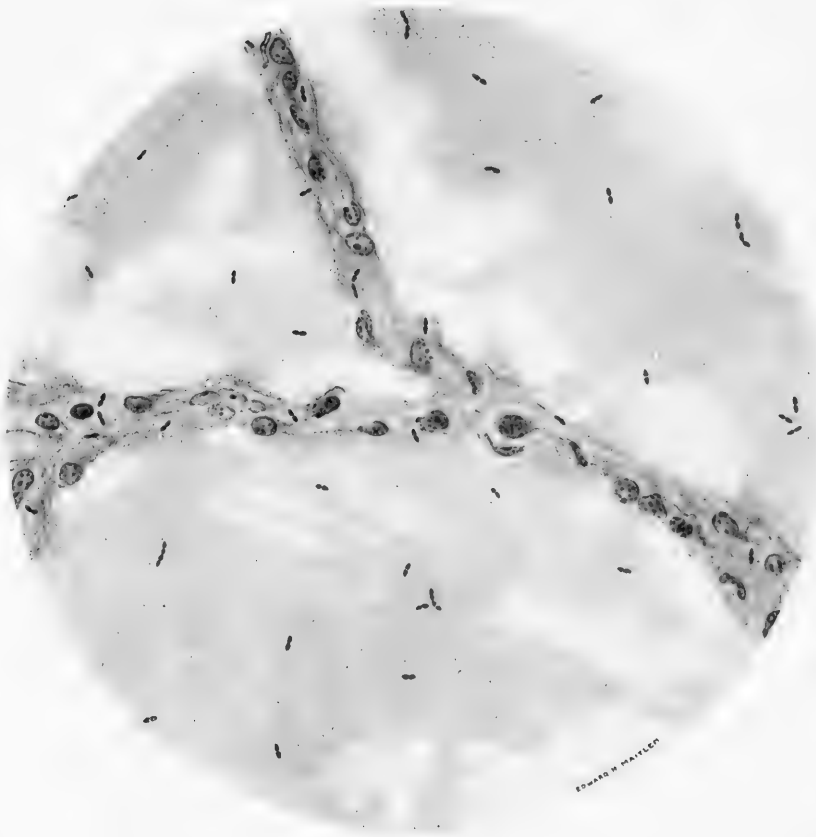

FIG. 26

(Blake and Cecil: Experimental pneumonia. II.)

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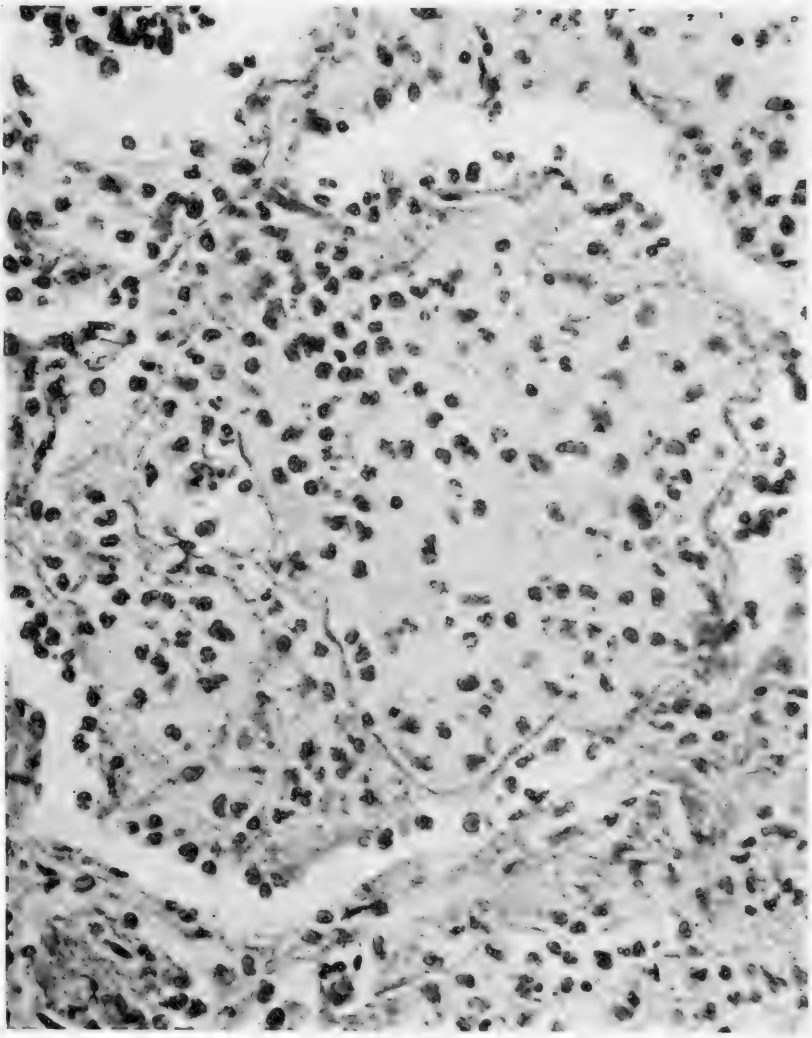

FIG. 27.

(Blake and Cecil: Experimental pneumonia. II.)

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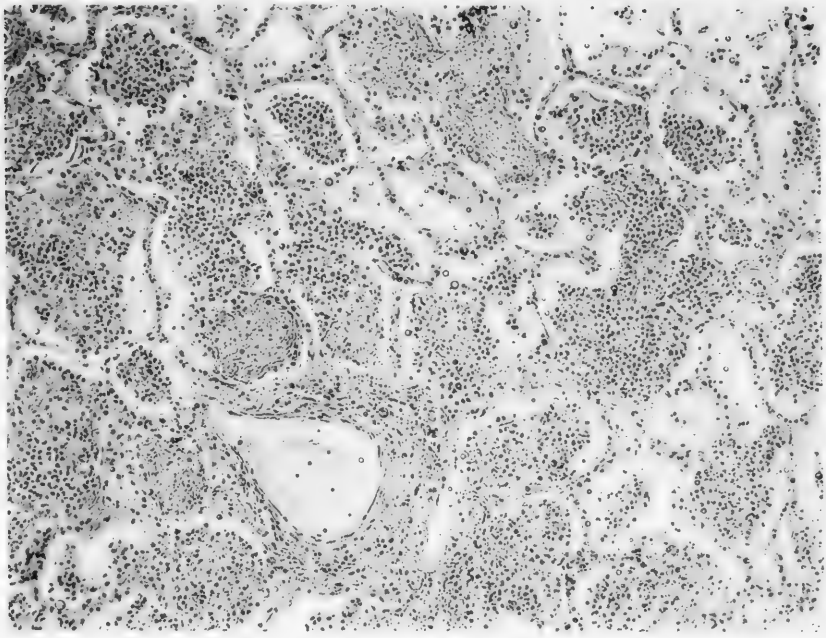

FIG. 28.

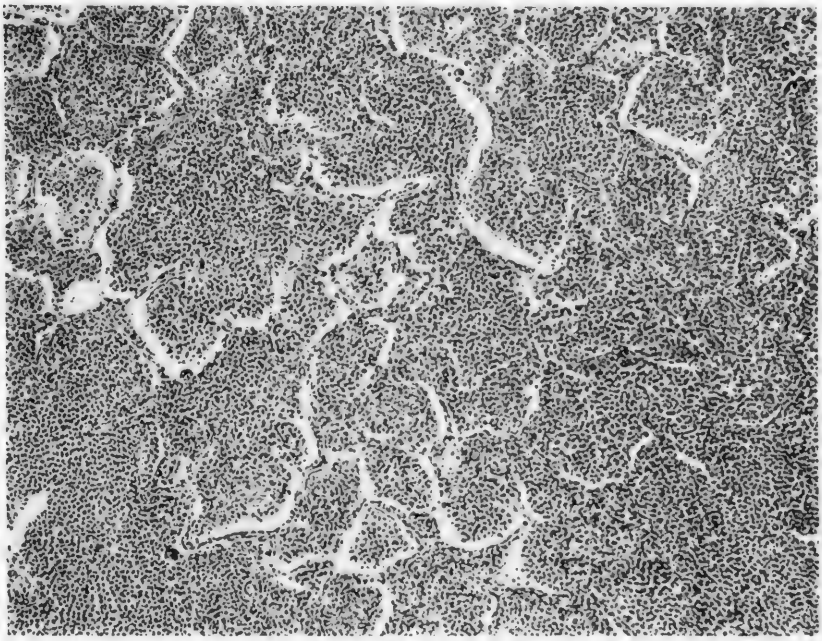

FIG. 29.

(Blake and Cecil: Experimental pneumonia. II.)

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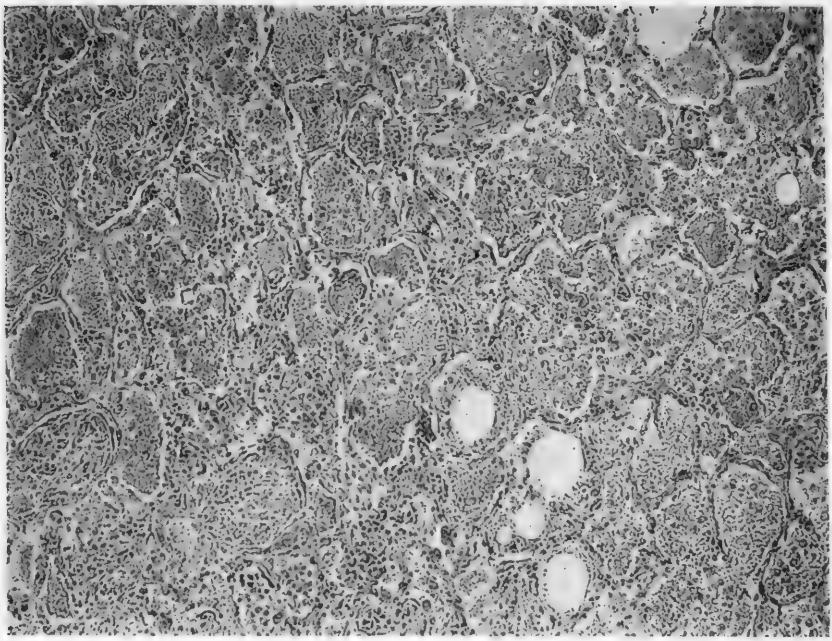

FIG. 30

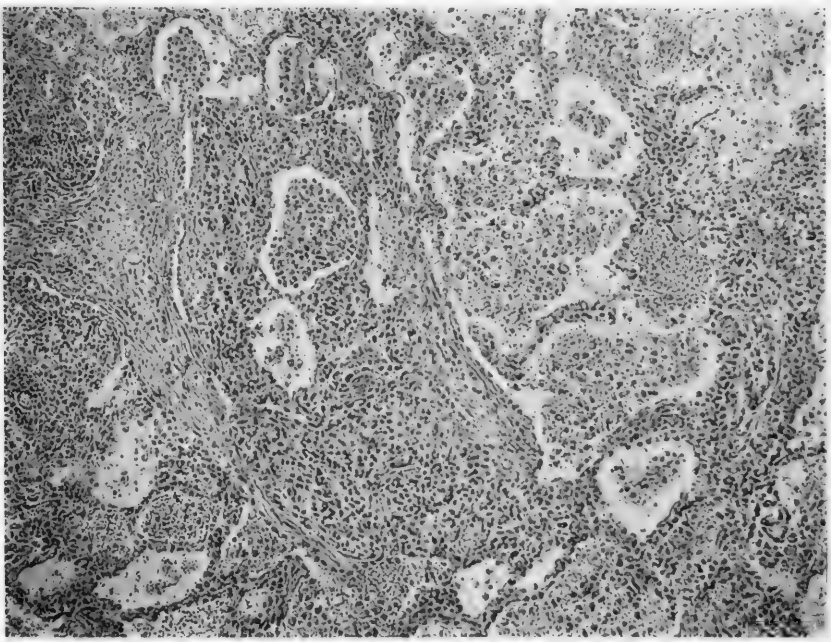

FIG. 31.

(Blake and Cecil: Experimental pneumonia. II.)

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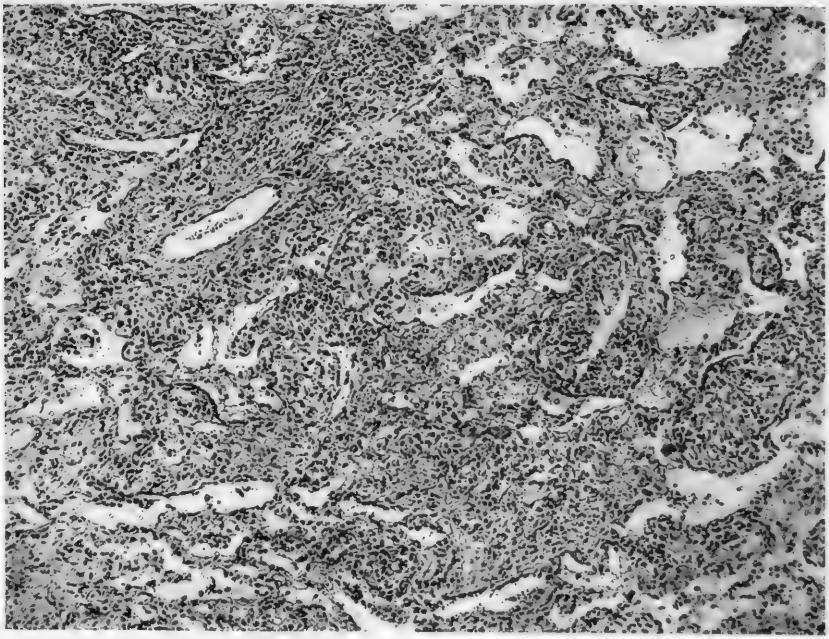

FIG. 32.

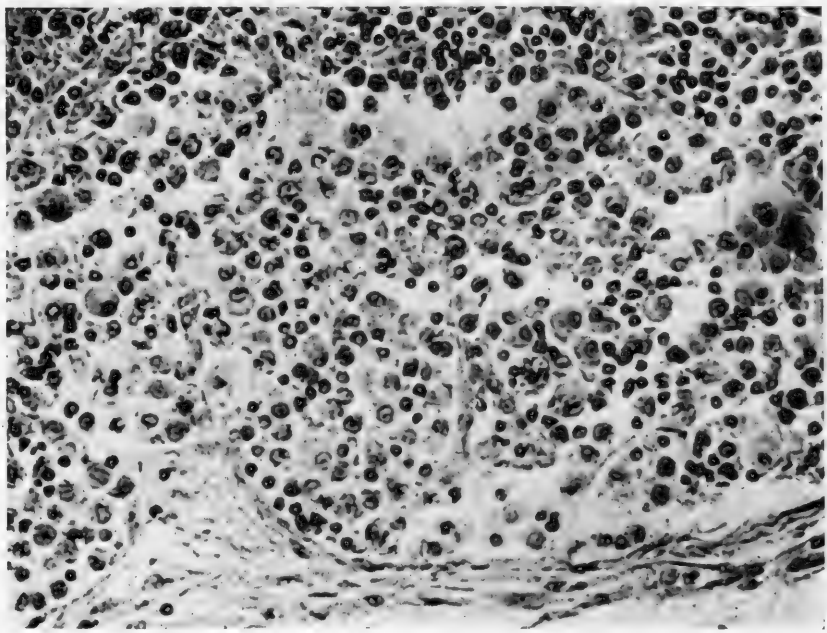

FIG. 33.

(Blake and Cecil: Experimental pneumonia. II.)

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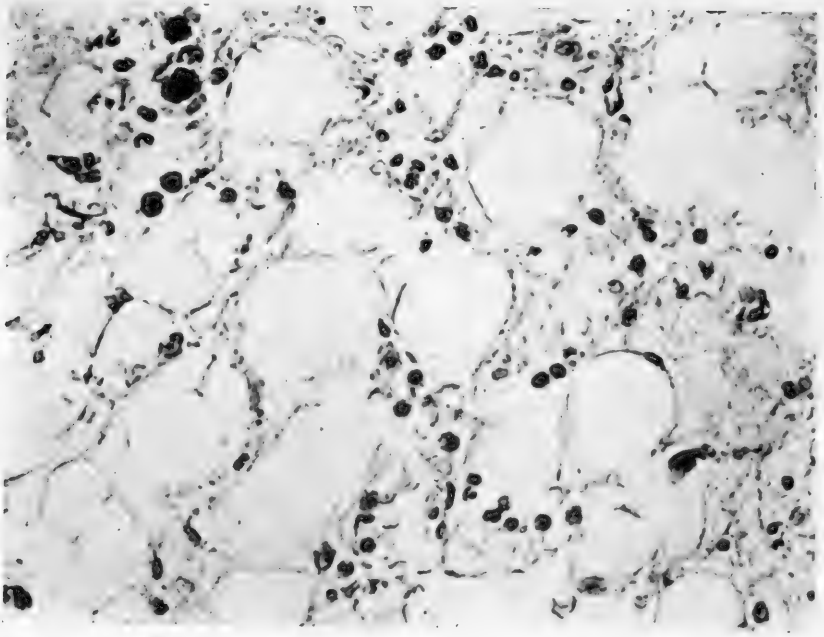

FIG. 34.

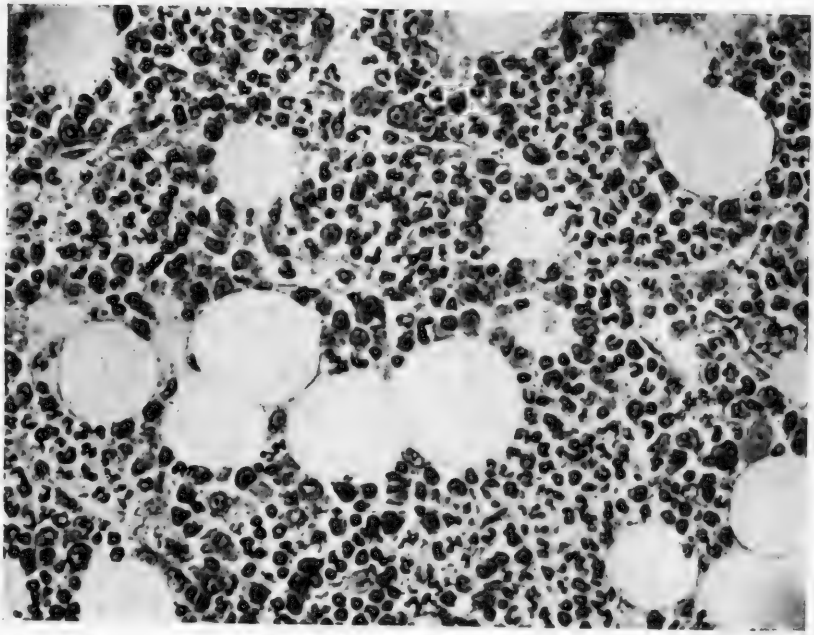

FIG. 35.

(Blake and Cecil: Experimental pneumonia. II.)

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EXPERIMENTAL SYPHILIS IN THE RABBIT.

I. Primary Infection in the Testicle.

By WADE H. BROWN, M.D., and LOUISE PEARCE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 45 to 52.

(Received for publication, January 29, 1920.)

The successful transmission of the virus of human syphilis to rabbits in 1906 (1) gave promise of an unusual opportunity for the investigation of problems relating to syphilitic infection by the use of experimental means, and during the years following, a great deal of time was devoted to the study of the animal infection with a view to adapting it to this purpose. Numerous methods of inoculation were devised and perfected and the resulting infections were studied in great detail, but the hope of obtaining an experimental infection by local inoculation which was closely analogous to the human disease was not fully realized. Isolated instances of generalized infection with the occurrence of lesions of various types have been reported from time to time, and while some investigators have obtained such evidence of generalization in as many as 50 per cent of the infected animals (2), these occurrences have been comparatively rare in the experience of most observers.

It should be remembered, however, that much of the work which has been reported was based largely upon the study of animals inoculated with virus recently isolated from human sources or with virus which had been carried in rabbits for only a few years, and that while most investigators are of the opinion that the virulence of *Treponema pallidum* for rabbits may be increased by passage or by adaptation, it is still uncertain how long this increase in virulence can be kept up or to what extent it may be carried.

The infection in the rabbit, as in man, is one which runs a prolonged course and is very variable in character, so that it would

necessarily require years of observation and comparison of infections in large series of animals to reach any definite conclusion upon these points. Thus far, it is doubtful whether any one has been in a position to carry out such experiments upon a scale which would enable him to say what form of disease might ultimately be established in the experimental animal or how closely this disease might be made to resemble the human infection. From the standpoint of the use of the experimental infection as a means of investigating problems of human syphilis, it is obvious, however, that the animal infection should be brought to a stage of development as near that of the human infection as possible. From our own investigations it appears quite likely that this condition is much more nearly attainable than results thus far reported might indicate.

Among the problems of human syphilis which have been attacked through the use of the experimental infection in the rabbit, that of therapy stands out as of foremost importance, and our interest in experimental syphilis grew out of the use of the rabbit infection as a means of studying the therapeutic action of drugs. While at no time have we been able to undertake the study of the experimental disease as an independent problem, we have, nevertheless, been forced to carry out a great deal of collateral investigation as the need for information of a given character arose. In addition, we have had the opportunity of studying a large series of animals infected with two strains of *Treponema pallidum* both of which have been carried in rabbits for a number of years.

What we have to report at present, therefore, is not the result of a series of experiments designed expressly for the solution of particular problems in experimental syphilis, but the results of a series of observations supplemented by experiments intended to give information of a particular character.

The chief needs for therapeutic work upon experimental syphilis are reliable means of propagating the infection and an accurate knowledge of the disease as it exists in the experimental animal. It is the purpose of these papers, therefore, to condense the results of 4 years observation into a series of brief reports dealing with clinical aspects of experimental syphilis in the rabbit with especial reference to the phases of the subject which are of importance in carrying out

therapeutic experiments. Detailed discussion of individual papers will be deferred until the series has been completed, when the various aspects of the subject will be considered together. Later, we hope to be able to take up other phases of experimental syphilis which cannot be included in the present group of papers.

It will be found that our description of the experimental infection in the rabbit differs materially from others in the literature, but this is not to be taken as evidence of conflicting observations. We have dealt with older strains of *Treponema pallidum* than have commonly been used, and different principles have been employed in the handling of these organisms, so that it is not unlikely that the infection produced by us has been of a different order. Whether or not one uses such organisms to begin with, it is well to have in mind the state of virulence to which an organism may be developed and the character of the infection which may be produced by these organisms.

Primary Infections in the Rabbit.

Three general forms of local inoculation have been successfully employed in the rabbit. These include inoculation into the anterior chamber of the eye, inoculation into the testicle, and inoculation of skin surfaces—usually the scrotum. Of these, the first is at present chiefly of historical and scientific interest. Testicular inoculation was introduced by Parodi (3) in 1907 and soon became the most widely used form of inoculation. It is generally recognized that testicular infections are especially well adapted to the maintenance of pure strains of the organisms, but they are on the whole of less value as objects of clinical study, since a large part of the process takes place in parts hidden from direct observation, and the infection is of a less stable character than that produced by skin inoculations. There are, however, certain features of the syphilitic reaction which are brought out to greatest advantage by this form of infection, so that the consideration of the experimental disease may logically begin at this point.

EXPERIMENTAL.

Organisms Used.—Two strains of *Treponema pallidum* were used in the work to be reported. One was obtained from Dr. Hans Zinsser and Dr. J. G. Hopkins and was isolated by them from a mucous patch on November 17, 1913, being Strain A of their series (4). The other organism was the Nichols nervous strain V for which we are indebted to Colonel M. A. Reasoner, Medical Corps, U. S. Army (2).

The first of these organisms has been carried in rabbits in our laboratory for 4 years and had been isolated 2 years before it came to us. The Nichols strain was isolated from the spinal fluid of a case of neurorecidive in June, 1912, and has, therefore, been carried in rabbits for more than 7 years.

Animals Used.—During the course of the work, many types of rabbits were used. There were many animals whose markings indicated either a pure stock or a strong admixture of such breeds as the Belgian hare, the Flemish giant, the Dutch belt, the Himalayan, the silver-gray, the Polish, the New Zealand red, the Angora, the English, and perhaps others. The majority of the animals, however, were of the common varieties of albino, gray, brown, brindle, black, blue or maltese, and animals of mixed or broken coloring. We have used animals of all ages and degrees of testicular development, from the young rabbit of 3 or 4 months in which the testicles were still but slightly developed to the very old rabbit with redundant scrotum and atrophic testicles.

From records showing the character of each rabbit used, its weight, testicular development, character of the scrotum, etc., it was found that in as far as the testicular infection alone was concerned, almost any type of animal with well developed testicles would give good results, but that if other features of the infection were to be considered also, the best results were obtained from the use of the small or medium sized albino and common gray or brown rabbits. As far as possible, animals were selected in which the testicles were well developed, with a preference for young rather than old animals.

Technique of Inoculations.—Testicular inoculations were carried out by the use of a virus emulsion prepared from an infected testicle or skin nodule. The animal which served as a source for the virus was anesthetized and the testicle or nodule excised with aseptic precautions. This material, or a portion of it, was then placed in a sterile mortar and finely minced with scissors, after which enough sterile salt solution (0.85 per cent) was added to moisten the entire mass. The material was then rubbed to a thick paste, more salt solution being added and thoroughly mixed with the contents of the mortar; the amount of salt solution to be added was determined by the dilution of the spirochetes in the emulsion as indicated by dark-field examination. The emulsion ordinarily used contained not more than two to three spirochetes to a microscopic field nor fewer than a single organism to two to three fields. Such an emulsion was usually obtained with from five to ten volumes of salt solution to one of tissue. The only object in attempting to control the strength of the emulsion was to insure

the presence of a sufficient number of organisms to produce a prompt reaction and to maintain some degree of uniformity in the dose of virus used in successive transfers.

The fluid emulsion was aspirated into a small glass syringe fitted with a 22 gauge needle ⅝ inch in length. The scrotum of the animal to be inoculated was sponged with 50 per cent alcohol, more for cleanliness than for antiseptic purposes, and 0.2 to 0.5 cc. of the emulsion was injected into the center of each testicle. The exact dose used was determined by both the strength of the emulsion and the size of the testicle to be inoculated. The precaution was taken to see that the needles used were sharp and smooth to avoid laceration and that the testicle was not unduly distended by the fluid injected.

This technique is one intended for intensive inoculations and never failed to produce a take. From time to time the procedure described was modified in several respects for different purposes, but we found it very simple of execution and entirely satisfactory for routine inoculations.

Material Studied.—From November, 1915, to September, 1919, 83 transfers of the two strains of *Treponema pallidum* were made by testicular inoculation. All the inoculated animals became infected and developed characteristic testicular lesions. Some were used for subinoculations and others for therapeutic experiments, but the course of the local infection was followed in a large number of animals from the time of inoculation to the spontaneous disappearance of the local lesions, and some animals were kept under constant observation for a much longer period of time.

Local Reaction to the Infection.

The infections produced in the testicles of rabbits inoculated with *Treponema pallidum* differed in many important respects. There were differences in the time and mode of onset and in the character and progress of the reaction as well as in the nature of the lesions developed, but on the whole the course of these infections tended to conform to a given plan and the variations were largely those of detail. The characteristic feature of the reaction observed in this series of animals was a tendency to the occurrence of a succession of changes separable into periods or phases of progression and quiescence or regression which gave to the local reaction a cyclic or relapsing course not unlike that which characterizes the infections produced by the blood spirochetes. This type of reaction occurred not only in the testicles but was the fundamental pattern of every phase of the syphilitic reaction in the rabbit, and forms, therefore, a logical basis for a consideration of syphilitic infections.

Incubation Period.

The time elapsing between inoculation and the development of testicular changes which could be recognized by external examination varied from 2 to 6 weeks, with occasional instances of longer or shorter periods of incubation. In fully 90 per cent of the animals, however, the incubation period fell within the narrower limits of from 3 to 4 weeks, and under properly chosen conditions of virus and rate of transfer, it was found that this time could always be brought within the 3 weeks period.

By dark-field examination of fluid obtained from the testicles, spirochetes were frequently demonstrated at a period well in advance of the appearance of clinical signs of infection. The same was found to be true of histological changes and not infrequently even gross alterations could be made out in the excised organ before any clinical sign of infection had become apparent. The time at which spirochetes and histological changes became demonstrable need not be reported in detail but was found to be within 7 to 10 days after inoculation.

Successive transfers did not necessarily shorten the incubation period to any appreciable extent. The dose of virus used, other things being equal, had a noticeable effect upon the speed of reaction, but this was not always the case. The vitality, or what may be termed the infectivity of the spirochetes, proved to be a factor of much greater importance than that of dosage. Thus, transfers made from actively developing lesions or from animal to animal as rapidly as the infection developed tended to produce or maintain a short incubation period, while inoculations made from old, inactive, or regressing lesions showed a relative prolongation of the incubation period irrespective of the dose of spirochetes used.

Mode of Onset.

The specific reaction in the testicle began in one of two ways, either as a small circumscribed focus of induration situated at the point of inoculation or as a diffuse swelling with increased tension of the entire testicle. When inoculations were made from old or inactive lesions or at relatively long intervals, the first form of reaction usually prevailed, but when transfers were made from one animal to

another in rapid succession, the reaction was more often of the second type. These differences in the character of the initial reaction were only temporary, as a rule, and tended towards a common type as the infection advanced.

Course of the Reaction.

Once the presence of a specific reaction became established, the progress of the infection was marked by certain changes such as enlargement and induration of the testicles which were common features of the reaction in all animals. The details of these changes necessarily varied in individual animals or even in the two testicles of the same animal, so that in general two extreme types of reaction with numerous modifications and variations became recognizable, depending upon the speed and sharpness with which successive changes occurred and the extent to which the several processes participated in the reaction.

In its highest form, the specific reaction in the testicle was characterized by a short incubation period, a diffuse onset and rapid development followed by a sharp crisis during which there was marked regression of the testicular lesions, a period of quiescence or inactivity, and one or more secondary cycles of active proliferation and quiescence or regression. Naturally, all these phases of the testicular reactions were rarely observed in their ideal form in one animal, but the several features of the reaction and some of its more common modifications may be illustrated by concrete examples.

Acute Exudative or Fulminating Reaction.—The first form of reaction to be cited is one which was characterized by an intense cycle of acute reaction terminating in crisis and followed by a slight secondary cycle of proliferative reaction. The case of orchitis shown in Figs. 1 to 6 furnishes an example of a reaction of this kind.

Fig. 1 shows the testicles at the time of inoculation. 15 days after inoculation, there was an increased tension and slight swelling of the testicles which progressed rapidly for about 6 days, the left testicle being more affected than the right (Figs. 1 to 3). At first, the scrotum was drawn tightly about the testicle, its vessels were slightly prominent, and there was a faint reddening of the skin surface (Fig. 2). On the 6th day, the reaction reached its height (Fig. 3), having

culminated in a diffuse congestion and marked edema of the scrotum most of which had developed within the last 24 hours.[1]

On the 7th day after the appearance of the specific reaction, there was a sharp change during which the congestion and edema of the scrotum subsided very rapidly and were followed somewhat more slowly by a decrease in the swelling of the testicle and a softening of the induration. Within 3 days, regression had reached the point indicated in Fig. 4. At the end of a week, the testicles had returned to approximately normal size and appeared as small, rather soft, atrophic masses with an area of thickening at the lower pole of the right testicle.

No further change was noted during the succeeding 10 days. Then on the 12th day, the mass at the lower end of the right testicle was found to be definitely enlarged and indurated. This marked the beginning of a second cycle of progressive reaction which at this time was limited to a circumscribed area in one testicle. 7 days later, the nodule in the right testicle had increased to nearly 1 cm. in diameter and a few small shotty nodules were palpable in the left testicle (Fig. 5).

The lesion on the right continued to develop for another week at which time it presented the appearance shown in Fig. 6. Meantime, the nodules on the left had disappeared completely and there was no further reaction in this testicle. During the next 2 weeks, the nodule on the right showed several short periods of quiescence and slight activity and then underwent complete resolution.

The difference in the intensity and extent of the reaction in the two testicles of this animal is of especial importance. It will be seen by comparing the photographs that the acute reaction in the left testicle was much more intense than that in the right, and that when the crisis came, regression in this testicle took place more rapidly and was more complete. In like manner, the secondary or proliferative reaction, while slight in both testicles, was decidedly less in the left than in the right. This relation between the intensity of the first cycle of reaction on the one hand, and the secondary reaction on the other was so constant as almost to establish a rule of inverse proportions between the first and second cycles of reaction in a given animal.

[1] At this stage of the infection, the scrotum and scrotal sac were filled with a gelatinous exudate or with a clear straw-colored or slightly blood-stained fluid which coagulated quickly on standing. The testicles also contained a fluid exudate of similar character which dripped freely when the organ was cut. Microscopic examination of these structures showed an inflammatory exudate composed chiefly of serum and fibrin with some polymorphonuclear leucocytes and red blood cells.

Chronic or Proliferative Reaction.—The second general type of reaction which occurred in the testicles of infected rabbits was one in which infiltration and acute exudative phenomena were subordinated to processes of proliferation. Reactions of this type showed, therefore, a more gradual onset and development of lesions with less marked cyclic alterations. In this group, the infection frequently began as a circumscribed focus of induration which gradually spread until the entire testicle became involved. Enlargement of the testicle took place more slowly as a rule than in the preceding group of cases and was associated with induration rather than increased tension of the testicle. The infection usually progressed steadily for upwards of 2 to 3 weeks by which time the testicle presented the appearance of a large, smooth or slightly nodular organ of extreme hardness. Congestion of the scrotum was comparatively slight, and edema, when present, was less marked than in the typically acute cases.

Instead of a pronounced crisis, progress of the reaction ceased more gradually and was followed by a period of inaction or of comparatively slow regression during which the testicles diminished in size and softened to a greater or less extent. If edema was present, it disappeared more rapidly, but at the end of the period of regression, the testicle still remained definitely enlarged and indurated. This residual induration was sometimes diffuse or uniform in distribution, while at others it was limited to certain areas or portions of the testicle.

Growth of the lesions was then resumed and continued with occasional remissions so that the progressive tendency of the reaction remained uppermost for a considerable period of time. The reaction in the testicles then subsided and the lesions resolved in much the same way as they had developed.

Two examples of this type of reaction are given in Figs. 7 to 9 and 10 to 12 which represent stages in the progress of the reaction in two animals of the same series. The incubation period in these two animals was 29 and 22 days respectively. In the first animal (Figs. 7 to 9), there were diffuse enlargement and induration which were first noted about 4 weeks after inoculation; the infection involved the scrotum as well as the testicles proper. The reaction reached its height in the left testicle 36 days after inoculation (Fig. 7) and in the right 39

days (Fig. 8). Both testicles then showed a slight regression (indicated in the photographs by a slight decrease in size) followed by renewed activity toward the end of the 7th week after inoculation (Fig. 9).

The reaction exhibited by the second animal differed somewhat from that of the first. There were again a diffuse enlargement and induration of both testicles, but, as indicated by a shorter incubation period, the reaction took place more rapidly, reaching its height during the middle of the 4th week after inoculation (Fig. 10). At the time the reaction was nearing its height in the first animal, a decided regression had already taken place in the second (Fig. 11). This regression was more marked than in the first case, and a well marked second cycle of reaction was not apparent until towards the end of the 7th week. The character of this reaction is indicated in Fig. 12 which shows the condition present 8 weeks after inoculation.

Variations in the Specific Reaction.
Subacute Reactions.

The modifications and combinations of these two fundamental types of reaction were, as we have said, quite numerous, but a few specific examples will serve to indicate the character and direction of the more important variations.

The first variation which may be mentioned is one concerning the acute type of reaction. The photographs reproduced in Figs. 13 to 15 illustrate a reaction which was characterized by an acute cycle of moderate extent associated with some diffuse induration in the testicle which persisted after the crisis. The reaction began in this animal as a circumscribed focus of induration in the posterior portion of the testicle, which was first detected 18 days after inoculation. Induration spread and the testicle enlarged rather rapidly, at the same time becoming diffusely indurated. The height of this change was reached 26 days after inoculation (Fig. 13). There was a moderate edema of the scrotum which lasted for a few days and then subsided, leaving the testicle still slightly enlarged as indicated in Fig. 14. There was some further regression of the lesions, and renewed activity did not set in until towards the end of the 7th week. This second cycle of reaction was both focal and diffuse in character and at the time the third photograph of the series was taken (Fig. 15) there were diffuse induration of both testicles and numerous nodules varying from a few mm. to approximately 0.5 cm. in diameter.

A second type of orchitis which was quite common in our series of animals is shown in Figs. 16 to 18. The onset of the infection in this instance was again of a circumscribed nodular character with an incubation period of 27 days. The testicles became diffusely indurated and showed a marked enlargement. This reaction took place somewhat more slowly than in the preceding case and did not

reach its full development until about 6 weeks after inoculation (Fig. 16). At the time the first photograph was taken (Fig. 16) the left testicle was retracted within the abdominal cavity and could not be brought through the inguinal canal, and there was very marked edema of both scrotal sacs which is best shown on the right. Edema subsided rather slowly, and at the same time the testicles diminished somewhat in size and became slightly softened. On the 49th day (Fig. 17) the scrotum showed a diffuse thickening with beginning induration over the ventral surface of both testicles; the testicles themselves were still considerably enlarged and markedly indurated. From this point onward, the reaction gradually shifted from the testicles proper to the scrotum with the production of chancre-like lesions, the beginning of which is indicated in Fig. 18.

The feature of especial interest in the reaction exhibited by this animal was the slowly progressive but pronounced character of the reaction, culminating in a diffuse swelling and edema of both the testicles and the scrotum and the subsequent transference of the center of reaction from the testicles to the scrotum—the latter condition being a frequent occurrence in testicular infections.

Another group of photographs illustrating an infection of somewhat the same character is reproduced in Figs. 19 to 22. This animal was an old albino with rather atrophic testicles and, as is usually the case with such animals, the reaction was slow to develop. Infection began as a circumscribed focus of induration which was recognized about 4 weeks after inoculation and gradually spread until the entire testicle was diffusely involved. There was a slow but steady increase in the size and induration of the testicles, extending over a period of about 2 weeks. The height of the reaction in the right testicle was reached 46 days after inoculation (Fig. 19) and a few days later in the left. There were moderate congestion and edema of the scrotum which lasted for several days. Crisis occurred, followed by regression, but at the end of this phase of the reaction, both testicles were still diffusely enlarged and indurated much as they appear in Fig. 20. Within 10 days after the crisis, there was renewed growth of lesions situated in the tail of the epididymis (Fig. 21). These lesions grew actively for another 2 weeks at which time they formed large indurated masses of irregular shape, involving the skin as well as the epididymis (Fig. 22). In the meantime, the reaction in the remaining portions of the testicle had completely subsided, leaving the testicles as small atrophic masses.

A fourth example of a more unusual reaction is given in Figs. 23 to 25. The earlier stages of the reaction in this animal were of an ordinary chronic proliferative type and had progressed to the point of the formation of a scrotal chancre on the left associated with induration of the testicle itself with marked enlargement and induration of the right testicle and slight extension to the scrotum at its lower pole, when, towards the end of the 7th week, there was an acute exudative reaction in the right testicle and scrotum, which reached its height on the 53rd day after inoculation. This reaction was followed by a typical crisis (Figs. 23 and 24), and both testicles then began to diminish in size and induration,

while the lesions of the scrotum continued to grow actively (Fig. 25). The feature of especial interest here was the occurrence of an acute exudative reaction late in the course of infection.

This small group of cases, including the more acute and chronic forms of reaction together with various modifications and combinations of these two fundamental types of processes, is typical of the reactions seen in practically all cases of outspoken testicular infection. Many of these modified reactions might be spoken of as subacute in the same sense in which the others are acute or chronic in character, since they combine to a greater or less degree the features of exudation and infiltration with those of proliferation of fixed tissue cells. It will be noted, however, that whatever the variation in the response of the individual animal, they all show an unmistakable tendency towards a reaction of a recurrent or a relapsing type.

It was found that these reactions could be influenced to a considerable extent in several ways. Thus, reactions of the more acute type were especially frequent when transfers were made from one animal to another in rapid succession or during the ascending phase of the acute reaction. In a small proportion of animals, the local infection terminated with a single cycle of reaction such as that described. As a rule, however, the acute cycle of reaction was followed by other changes leading to the formation of lesions differing in many ways from those originally produced. The occurrence of these secondary cycles of reaction depended to a considerable extent upon the character and extent of the first cycle. Thus animals in which there was an intense, acute reaction were, as a class, less apt to show secondary reactions of marked degree than those in which the process had been more gradual or less intense.

Reactions tending towards the chronic or proliferative type were quite common and occurred with especial frequency after inoculations made at long intervals or from chronic indurative lesions. They were frequently associated with the most persistent local infections and gave rise to some of the most conspicuous and destructive lesions of the testicles.

Late Developments of the Testicular Reaction and the Character of the Lesions Produced.

Most writers have treated the testicular infection of the rabbit from the standpoint of the lesions produced, but we prefer to regard these lesions more as manifestations of a reaction to infection and hence very little has been said concerning the lesions themselves. Following out their ideas of the importance of the lesion as an entity in itself, many investigators have attempted to differentiate between the various types of lesions produced, and several classifications have been proposed, based largely upon the location of the lesion and the character of the pathological process (5, 6). In our experience such differentiations would be very difficult to make except as applied to a particular lesion at a particular time or to what may be termed the residual lesions which ultimately come to be established.

Without entering into a detailed discussion of this phase of the subject, it may be said that while the infection in the animals studied by us began either as a circumscribed or as a diffuse process, neither of these conditions was permanent, and the same applied to the character of the pathological process. In practically all instances, the infection ultimately involved the entire organ with the production of lesions in the parenchyma, tunics, epididymis, and cord, and in many instances in the scrotum as well. This extension took place early, as a rule, and by the time the reaction had reached its height, there was what might be termed a panorchitis.

In many animals, this widespread involvement was of a perfectly uniform character as far as could be determined by the gross appearance of the organ or by palpation. In a second group of animals, there was a finely granular condition of the testicle, while in a third, distinct indurated nodules could be recognized, separated by tissue showing a lesser degree of involvement or an involvement of a different character. This differentiation into lesions of a particular type or lesions situated in certain localities came as a late development in the course of the infection and was, in our opinion, attributable to the character of the reaction against the local infection and the tendency on the part of the infection to extend in certain directions. During the later stages of the infection, therefore, one fre-

quently had to deal not with a widespread infection at the height of its activity but with an infection which was localized only in certain areas. The points of most frequent involvement were the globus minor and major, the tunics, the mediastinum testis, and the skin, or, in other words, the areas in which membranes or connective tissue in some form was most abundant.

These later developments and extensions of the testicular infections are of considerable interest both from the standpoint of the reaction to infection and the lesions which are produced. Several examples of this phase of the local reaction have already been given in the preceding illustrations, which may be supplemented by Figs. 26 to 37.

The first group of photographs in this series (Figs. 26 to 28) illustrates an infection which began as a diffuse indurative orchitis. At the period of infection represented in Fig. 26, 58 days after inoculation, a more or less diffuse extension of the infection to the scrotum of the right testicle had taken place, while on the left, the lesions in the testicle and scrotum were assuming a more nodular character, and these differences in the character of the changes in the two testicles persisted to the end. As the infection advanced, a large portion of the right testicle and scrotum underwent diffuse necrosis; on the left, the lesions began to be more circumscribed and two well defined scrotal lesions were formed (Fig. 27). Eventually, a large portion of both testicles underwent necrosis as indicated in Fig. 28, which shows the condition 80 days after inoculation. Even at this stage, however, the multinodular character of the lesions of the left testicle could still be made out, there being three fairly well defined centers of reaction.

In this animal, the feature of especial importance in the reaction was the tendency to widespread necrosis which eventually involved the entire skin surface and the testicle as well. This was not always the case, however, even when the reaction in the testicle was fully as marked as in this instance. In Fig. 29, a case of orchitis is illustrated in which the involvement of the testicle was also quite marked, but the necrosis and ulceration were confined to two more or less circumscribed areas producing effects more analogous to primary skin lesions. Lesions of this type were very common among our animals, and all gradations and transitions could be found between the lesion which was definitely a diffuse orchitis with necrosis and ulceration and lesions which were identical in character with primary skin lesions. The photographs reproduced in Figs. 30 to 32 are given to illustrate this point.

Fig. 30 shows a condition which is clearly an orchitis with skin involvement which has led to the formation of depressed ulcers with a definitely indurated collar such as is seen in primary skin lesions. In Fig. 31, taken only 6 days later, the picture has changed somewhat, and the whole mass in the left testicle

has filled out, while the right testicle now shows a single large ulcerated lesion identical with the large skin chancres which develop in the rabbit.

The next step in the series of transition is shown in Fig. 32. The infection in this animal began as a diffuse indurative orchitis which later became nodular with lesions such as those shown in Fig. 21, except that there was an active nodule situated in the tunic of the left testicle. The nodule in the epididymis of the right testicle extended to the skin, forming a chancre-like mass seen in Fig. 32. On the left, the nodule in the tunic was the one to develop most actively, forming a skin lesion still more like the ordinary chancre. These lesions healed in the course of about 4 weeks, leaving an active nodule in the epididymis of the left testicle (Figs. 33 and 34).

A final transition in the course of the testicular infection may be illustrated by Figs. 35 to 37. The infection in this animal began as a circumscribed focus of induration which quickly developed into a diffuse orchitis (Fig. 35). In time, the diffuse induration resolved, leaving small focal lesions in the tail of the epididymis of both testicles. Foci of infection then appeared in the skin and developed into the lesions shown in Fig. 36 (77 days after inoculation). Upon removal of the testicles and skin lesions, the testicles were found to be small atrophic masses entirely free from any gross evidences of active infection (Fig. 37). On the right, there was an indurated lesion in the epididymis which in part was continuous with the skin lesion; on the left, the two processes showed less connection and one of the two lesions in the scrotum was entirely distinct from the other lesions present (Figs. 36 and 37).

In this animal an infection which began as a circumscribed focus of induration first became diffuse, was then transformed into a nodular epididymitis, and the seat of active infection was finally transferred to the scrotum, leaving the body of the testicle free from active lesions.

Atypical and Low Grade Reactions.

Before leaving the subject of the local reaction in the testicle, mention should be made of another class of infections. There were, in our series, a few animals which showed some peculiarity in their response to infection, such as the development of slight or otherwise atypical lesions. These infections may be spoken of as atypical, on account of such peculiarities in the reaction, or as low grade reactions, in the sense that the reaction lagged or that the lesions which developed were of a minor character.

The common instances of this kind were slight reactions of short duration and slight but persistent reactions. Of the latter class, there were two main groups of infections, one in which a diffuse or

circumscribed focus of reaction developed very slowly and never resulted in more than a slight diffuse thickening in the testicle or a small nodular induration. The second group included instances in which a slight diffuse reaction subsided with the formation of residual nodules or in which an initial focus of infection was later supplemented by the appearance of secondary foci of a similar character. Minor lesions such as these not infrequently persisted for months without showing any especial sign of activity or growth.

Most of the infections of this class occurred during the earlier part of the work and have rarely been seen during the last 2 years. We are inclined to attribute occurrences of this kind to the circumstances under which the transfers were made.

From our present knowledge of conditions which influence the specific infection in the rabbit, we recognize this class of cases as attributable to certain definite causes, mainly to an attempt to inoculate with spirochetes at a time when their vitality, or infectivity, had been materially reduced by cyclic immunological reactions (page 492), but also to peculiarities in the response of individual animals to the specific infection.

Accessory Skin Lesions.

For the sake of completeness, mention may be made also of the occurrence of accessory lesions in the scrotum which develop along the path of the needle as a direct result of the process of inoculation (Figs. 2 and 4). At times, these lesions appeared before any reaction in the testicle had become recognizable. Without going into a discussion of this feature of testicular infections, which properly belongs in the section on scrotal chancres, it may be said that these foci of infection appeared in one of three forms, as small gelatinous swellings in the skin, as translucent pearly nodules in the superficial layers of the skin, or as opaque points or plaques in the depths of the scrotum, usually upon the outer surface of the tunica vaginalis. They rarely developed to lesions of any considerable extent. In some instances, however, they did develop into large lesions either as independent foci of reaction or as indistinguishable parts of the testicular reaction.

The Spirochete Reaction and the Spirochete Content of Lesions.

The above observations upon the course of the specific reaction in the testicle were supplemented by parallel dark-field examinations of fluid taken from the testicular lesions at different periods of the infection in order to determine what changes, if any, could be demonstrated among the infecting organisms while the changes in the testicles were in progress. From these examinations, it was found that as the infection progressed, the spirochetes exhibited changes analogous in character to those described in the testicles themselves. As we have already mentioned, spirochetes began to multiply and were present in considerable numbers before any gross manifestation of infection had become recognizable. This increase in spirochetes continued parallel with the development of the lesions, so that by the time the lesions had reached the height of their first cycle of development, actively motile spirochetes were present in large numbers.

At this point, the spirochetes suddenly began to lose their motility and to collect in tangled masses. In cases of acute orchitis, these changes coincided roughly with the development of edema of the scrotum and were followed promptly by regression of the testicular lesions.

Following the phenomenon of agglomeration, the spirochetes rapidly diminished in numbers, so that within a few days, organisms were difficult to find in fluid aspirated from the testicles, and those seen were either degenerated or showed but slight signs of motility; in many instances no organisms could be found.

After passing through a crisis such as this, actively motile spirochetes again appeared in the testicular fluid, and increased in numbers, presaging a renewed activity on the part of the lesions or at least a cessation of regression for the time being. These parallel changes continued throughout the existence of the local infection—the change in spirochetes usually occurring slightly in advance of the changes in the lesions.

As in the case of the lesions, cyclic changes were at times very sharp and easily recognizable, while at others they were less marked and might readily have escaped detection had it not been for the example of the sharper type of reaction.

During the latter part of the infection, the spirochetal content of lesions was found to be more difficult of estimation and somewhat uncertain. Where a single lesion was present, diffuse or circumscribed, the spirochetes in one area might diminish or disappear while they were present in considerable numbers or were actively increasing in other areas. These changes in the spirochetal content had their parallel, however, in the shifting centers of growth in the lesions themselves or, in the case of multiple lesions, in the resolution of one lesion while another was undergoing active development. By careful study of the lesions, it was found, however, that actively growing lesions or portions of lesions always contained actively motile spirochetes, and the same was true of many lesions which were merely quiescent but not regressing. The spirochetal content of lesions which were regressing was more variable; in many instances, spirochetes could not be detected by dark-field examination, while in others, they could still be demonstrated in fair numbers which diminished as the lesions resolved.

The changes affecting the spirochetes within the lesions will be recognized as entirely analogous to those which occur in blood stream infections with such organisms as *Spirochæta recurrentis*. In order to assure ourselves that the apparent immobilization, agglomeration, and degeneration described, entailed some actual alteration in the pathogenic properties of these organisms, tests were carried out by animal inoculation. For this purpose, inoculations were made with organisms taken as nearly as possible at the height of the first cycle in cases of intense, acute reaction, or more properly at the beginning of the crisis, and the tests were controlled by a parallel series of inoculations made with actively motile spirochetes taken from an early stage of testicular infection.

From these tests it was found that the infecting power of such organisms was markedly diminished. In one experiment, a series of animals inoculated with approximately ten times the dose of immobilized and agglomerated organisms that was used in the controls showed an incubation period of 6 weeks as contrasted with 3 weeks in the controls, while the lesions were slow to develop and were less pronounced than in the control animals. Similar observations as to the infectivity of the spirochetes at different periods of the infection have

been made many times and there is no doubt in our minds as to the significance of the cyclic reactions described.[2]

Duration of the Local Infection.

The duration of the local infection as determined by the presence of active lesions was as variable as the course of the infection itself, and no fixed limits can be given either for the several phases of the local reaction or for the infection as a whole. The period of active infection varied anywhere from 1 to more than 12 months. In some animals, the entire reaction was represented by one intense cycle of acute reaction which terminated within 4 to 6 weeks after inoculation; in others, the infection continued through successive cycles of reaction, but the period of active infection was rarely longer than 2 to 4 months. Inactive or latent lesions which showed occasional periods of slight activity frequently persisted much longer, and residual lesions in the epididymis, skin, and tunics not infrequently persisted for from 4 to 8 months. In general, the duration of the local infection was inversely proportional to the intensity of the local reaction.

CONCLUSIONS.

The conclusions which might be drawn from this series of observations are very numerous, but we shall refer briefly to only a few of the more important points.

From the standpoint of a pathological process, it is important to note that the local response is not altogether a granulomatous reaction. In fact, it appears that exudation and infiltration are the fundamental processes and that proliferation is a secondary phenomenon.

In the second place, it is quite clear that the reaction to infection in the testicle, and hence the course of the infection itself, are of a periodic or relapsing character, analogous in this respect to other spirochete infections, or for that matter present certain protozoan

[2] We have evidence sufficient to indicate that rabbits inoculated in the testicles or scrotum with *Treponema pallidum* always show a blood stream invasion, and that these organisms are subject to changes analogous to those which occur in the testicles. The details of this work will be reported later.

characteristics. To what extent this infection is influenced by a local reaction and to what extent by a systemic reaction are at present unknown, but as far as the local infection is concerned, it appears to be subject more to local than to general conditions. Further than this, it appears that the character of the reaction which takes place in a given case is itself significant. The relation between the reaction in the individual animal and the duration of the infection represents a constant. When the local reaction assumes the character of a chronic proliferative process, the life of the infection is prolonged and the extent of the reaction which takes place before the infection is brought under control is proportionately increased; when, however, the reaction assumes the form of an intensely acute reaction, the life of the local infection is promptly terminated. This relation between the local reaction and the duration of an active infection, together with the determination of the character of the experimental infection in the rabbit, are perhaps the two most important deductions to be drawn from this series of observations in that they touch every phase of the experimental infection.

SUMMARY.

A study was made of the infections produced in rabbits inoculated in the testicles with two strains of *Treponema pallidum* which had been carried in rabbits for several years. Infection resulted in all instances; the incubation period varied as a rule between 2 and 6 weeks and under properly chosen conditions could be reduced to approximately 3 weeks or less.

The resulting infection pursued a typically cyclic or relapsing course which affected both the spirochetes and the associated lesions in the testicle. The spirochetes in the local lesions exhibited periodic changes less marked and less regular but identical in character with the changes which occur in the blood in cases of relapsing fever. The lesions in the testicle also showed periods of active development and quiescence or regression which followed closely upon the changes exhibited by the spirochetes.

The specific reaction in the testicle showed considerable variation in the speed and sharpness with which successive phenomena occurred

as well as in the character and extent of the processes themselves. These reactions were of two fundamental types. In one group of animals, the reaction was characterized by an intense cycle of acute exudation and infiltration with a lesser degree of proliferation, followed by crisis and subsequent recurrence of secondary cycles of proliferative reaction of a minor degree. In the other group of animals, the reaction was more chronic in character and consisted largely of infiltration and proliferation. The progress of the reaction was more gradual, and sharp alterations in its course were absent. The infection progressed by a succession of stages with slight and irregular remissions.

In a third group of animals, the reaction was subacute, combining at the same time the processes of exudation, infiltration, and proliferation. The first cycle of reaction was fairly acute and terminated in a definite crisis with moderate regression which in turn was followed by recurrence and more or less pronounced secondary cycles of proliferation.

In all cases of outspoken infection, there was diffuse involvement of testicle, tunic, epididymis, and cord, but as the infection progressed, the lesions underwent many transformations, so that a variety of lesions was formed from processes which in the beginning were of a common type. Eventually, the reaction became more irregular and the infection became centered in one or more foci which were commonly situated in the epididymis, tunics, scrotum, or mediastinum testis. These centers served as residual foci of infection.

The duration of the testicular process was found to be very variable. In some animals, the entire reaction consisted of but a single sharp cycle, and the local infection was terminated by crisis within 4 to 6 weeks after inoculation. As a rule, the period of active infection was from 2 to 4 months, and quiescent or inactive lesions not infrequently lasted for from 4 to 6 months. In exceptional instances, local infection persisted for more than a year.

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EXPLANATION OF PLATES.

The figures are reproductions of untouched photographs which represent the objects at their natural size. The statements of time refer in all instances to the time after inoculation, unless otherwise stated.

PLATE 45.

FIGS. 1 to 6. An acute diffuse reaction in the testicles and scrotum with crisis and regression followed by a slight secondary cycle of reaction and the formation of circumscribed nodular lesions.

FIG. 1. The normal testicles.

FIG. 2. 20 days. Well advanced, acute orchitis with beginning congestion and edema of the scrotum.

FIG. 3. 24 hours later. The height of the first cycle of reaction with intense congestion and edema most marked in the left testicle.

FIG. 4. 72 hours after the crisis. Note the greater regression of the left testicle.

FIG. 5. 48 days. The second cycle of reaction and the formation of nodular lesions in both testicles.

FIG. 6. 56 days. The lesion in the tail of the epididymis and scrotum of the right testicle is still increasing, while the lesions have disappeared from the left.

PLATE 46.

FIGS. 7 to 9. Chronic proliferative orchitis with scrotal involvement.

FIG. 7. 36 days. The height of the first cycle of reaction in the left testicle. Note the perfectly uniform enlargement of the testicle extending even to the cord. The scrotum of both testicles is involved, and on the right, there is an area of beginning necrosis.

FIG. 8. 39 days. The height of the first cycle of reaction in the right testicle; the crisis in the left has passed and the lesions are regressing.

FIG. 9. 51 days. The second cycle of reaction in progress in both testicles. Note the smooth, tense scrotum of the right testicle indicative of diffuse involvement, while in the left the lesions are now assuming a multinodular character.

FIGS. 10 to 12. Chronic proliferative orchitis in another animal of the same series as that in Figs. 7 to 9.

FIG. 10. 24 days. Well marked diffuse induration of both testicles approximately at the height of the first cycle of reaction.

FIG. 11. 36 days. Decided regression of the lesions of both testicles.

FIG. 12. 56 days. An early stage of the second cycle of reaction.

PLATE 47.

FIGS. 13 to 15. An acute diffuse reaction of moderate degree followed by crisis and a second cycle of chronic proliferation, diffuse and focal in character.

FIG. 13. 26 days. The height of the acute reaction with moderate edema of the scrotum. The left testicle is larger than the right.

FIG. 14. 28 days. Regression. The left testicle is still larger than the right due to a greater degree of proliferation in the initial reaction. Compare Figs. 13 and 14 with Figs. 3 and 4.

FIG. 15. 53 days. Moderate enlargement and induration of both testicles.

FIGS. 16 to 18. Subacute orchitis characterized by marked induration and edema of both testicles and scrotum and gradual shifting of the center of reaction from the testicles to the scrotum.

FIG. 16. 41 days. The left testicle is retracted within the abdominal cavity.

FIG. 17. 49 days. Some regression has taken place, but the scrotum is thickened and both testicles are still diffusely enlarged and indurated.

FIG. 18. 52 days. The lesions in the testicles are still slowly diminishing, but focal lesions are beginning to develop in the scrotum.

PLATE 48.

FIGS. 19 to 22. A subacute reaction with diffuse involvement of both testicles followed by a second cycle of reaction localized in the tail of the epididymis.

FIG. 19. 46 days. The height of the first cycle of reaction in the right testicle.

FIG. 20. 53 days. The extent of the regression following the crisis in the reaction is shown. Note that at the lower end of the right testicle, there is a nodule which has not regressed to the same extent as the rest of the testicle.

FIG. 21. 60 days. Continued regression of the testicular lesions with development of focal lesions in the epididymis.

FIG. 22. 76 days. Focal lesions of the epididymis and scrotum.

PLATE 49.

FIGS. 23 to 25. An acute exudative reaction occurring during a late stage of the local infection.

FIG. 23. 53 days. Chronic proliferative changes in both testicles with localized lesions of the scrotum with acute congestion and edema on the right.

FIG. 24. 2 days later. Regression on the right with slight swelling of the testicle and increasing growth of the scrotal lesion on the left.

FIG. 25. 61 days. Regression of the testicular lesions with simultaneous growth of the lesions in the scrotum of both testicles.

PLATE 50.

FIGS. 26 to 28. Chronic orchitis with extensive necrosis of testicles and scrotum. Right diffuse; left nodular.

FIG. 26. 58 days. Diffuse induration of the right testicle and scrotum with beginning skin necrosis and multinodular lesions of the left testicle.

FIG. 27. 73 days. Diffuse necrosis of the right testicle and scrotum; focal necrosis of the left.

FIG. 28. 80 days. Later stage of same lesions. Note lines of demarcation between the three masses on the left.

PLATE 51.

FIG. 29. Chronic diffuse orchitis with circumscribed lesions of the skin resulting from metastatic infection of the testicles.

FIG. 30. Chronic diffuse orchitis with multiple chancre-like lesions of the skin.

FIG. 31. Same testicles 6 days later showing transformation of the lesions. Note especially the single large chancre-like mass in the right testicle.

FIGS. 32 to 34. Late transformations in a case of testicular infection.

FIG. 32. Chancre-like extensions to the scrotum.

FIG. 33. Healing of the skin lesions with an active nodule persisting in the epididymis of the left testicle.

FIG. 34. 93 days later. Skin lesions practically healed, while the nodule in the epididymis is still active.

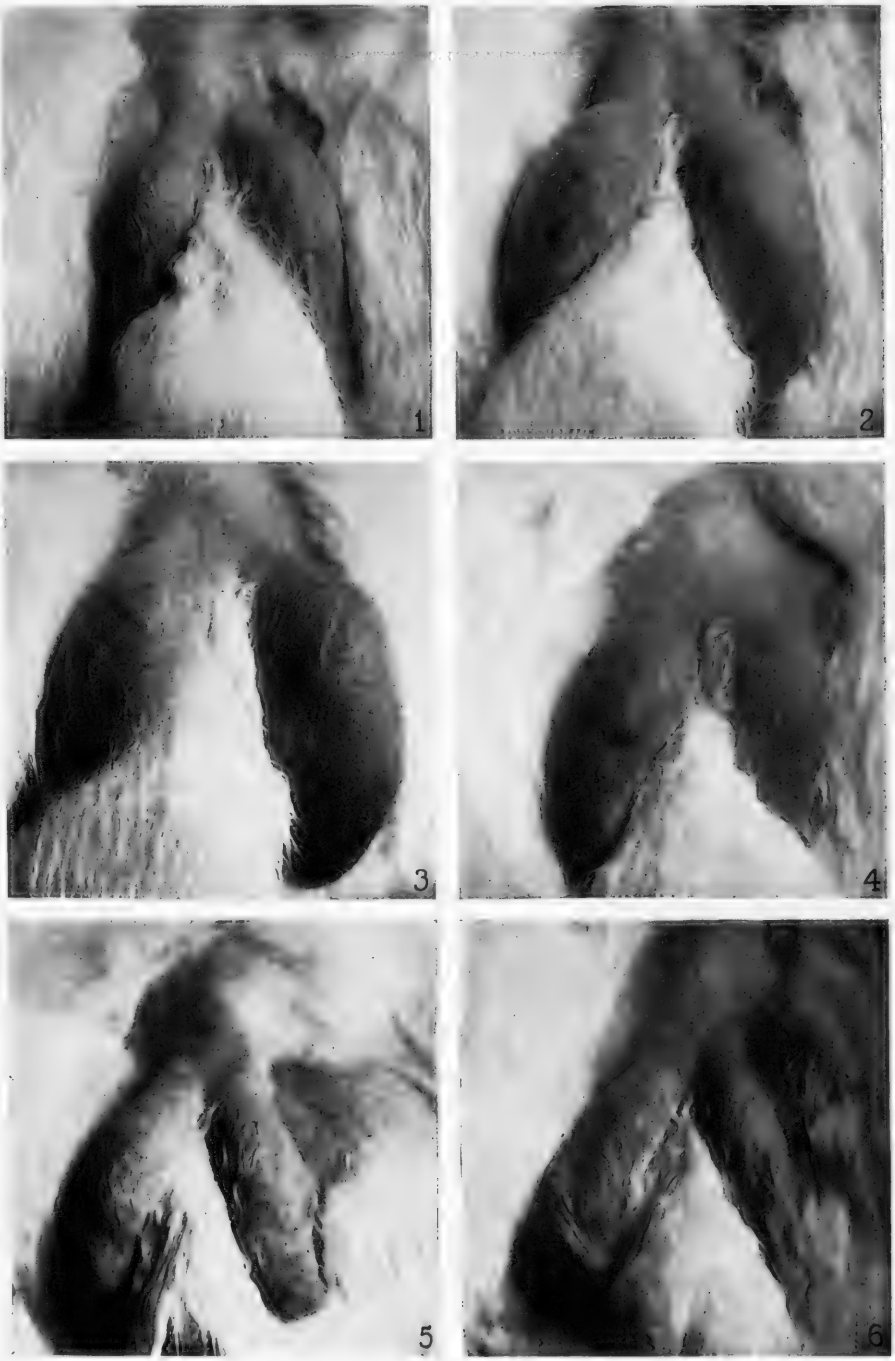
PLATE 52.

FIGS. 35 to 37. Diffuse orchitis with eventual transference of the local infection from the body of the testicles to the epididymis and scrotum.

FIG. 35. Diffuse indurative orchitis of both testicles.

FIG. 36. The final lesions in the epididymis and scrotum.

FIG. 37. The same lesions excised and sectioned. Both testicles are atrophic; on the right, the nodule in the tail of the epididymis fits into a hollow beneath the ulcer in the skin, but the two lesions are not entirely fused with each other; on the left, there is only a small nodule in the epididymis which is fairly distinct from the two lesions in the scrotum.



(Brown and Pearce: Experimental syphilis in the rabbit. I.)

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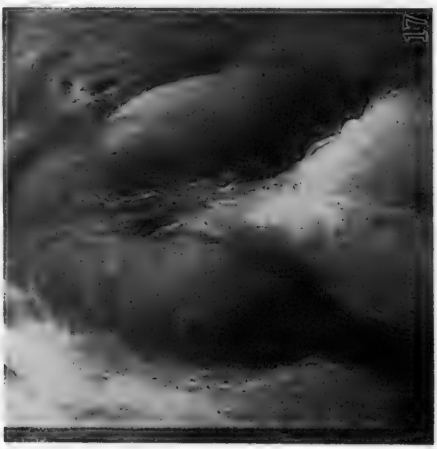

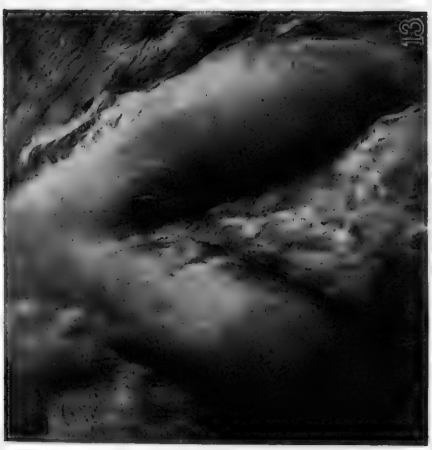

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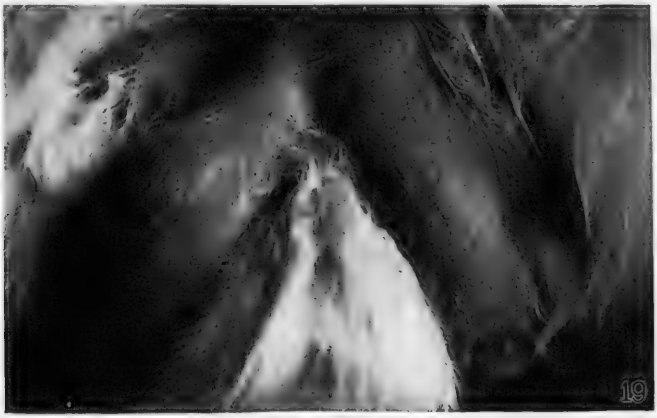

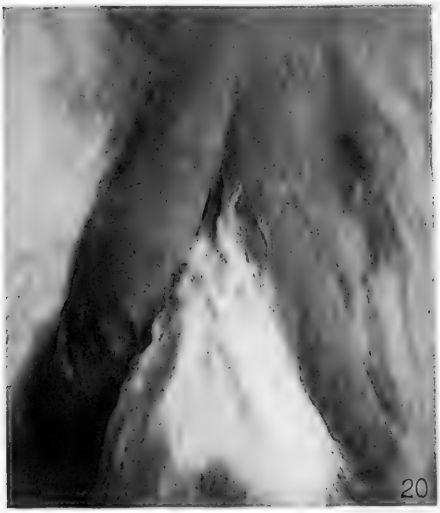

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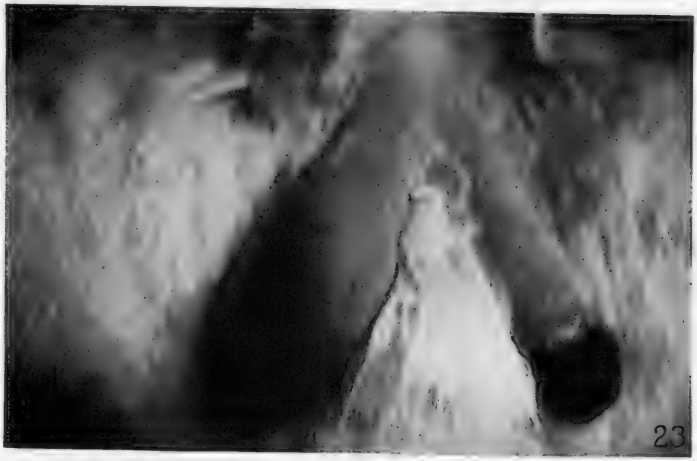

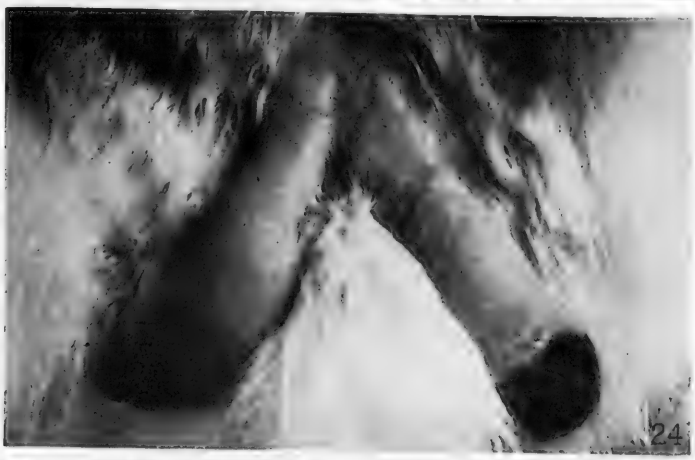

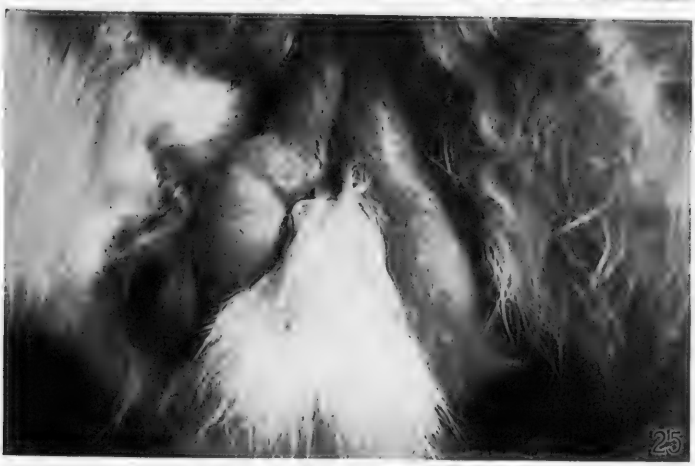

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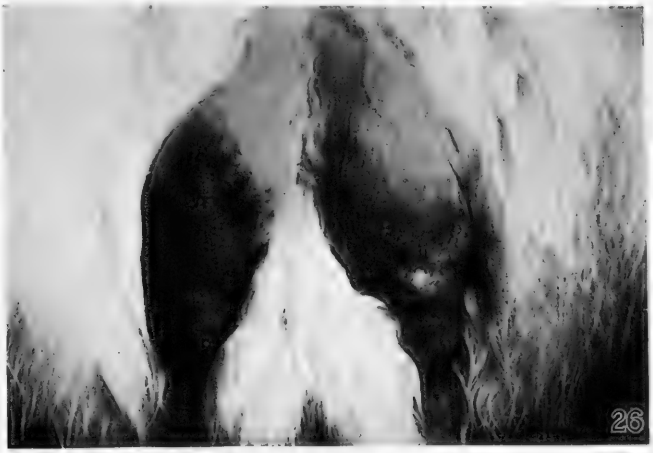

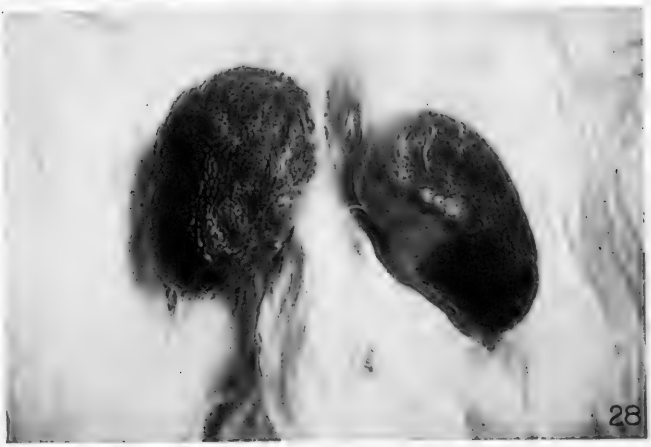

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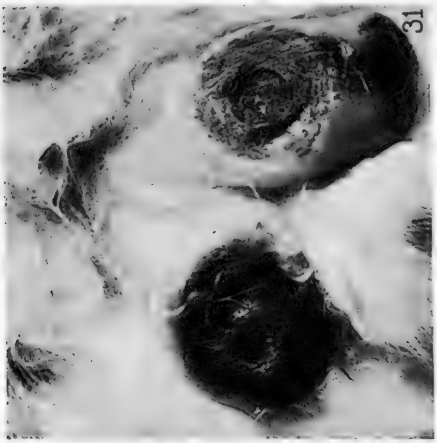

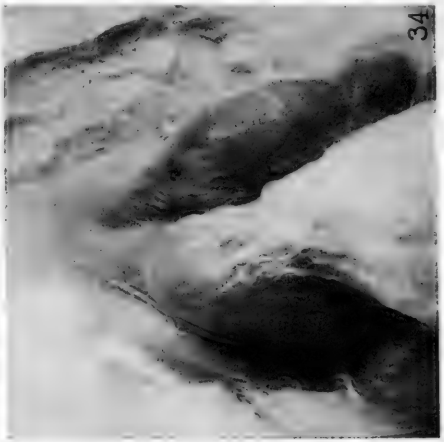

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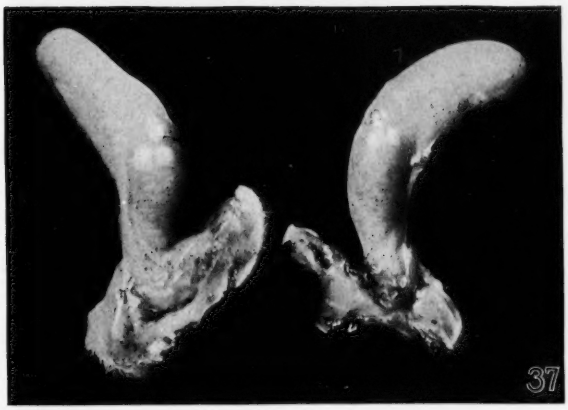

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